EFFECT OF MONENSIN SUPPLEMENTATION ON FOLLICULAR DEVELOPMENT OF BRAHMAN COWS AND THE EFFECT OF SEX AND TEMPERAMENT ON RESPONSE OF BEEF CALVES TO *SALMONELLA* NEWPORT EXTRACT VACCINE

A Thesis

by

ANITA MAE SNELL

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Chair of Committee,	Jason P. Banta
Co-Chair of Committee,	Thomas H. Welsh, Jr.
Committee Member,	Ronald D. Randel
Head of Department,	H. Russell Cross

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ABSTRACT

Beef production relies on the intricacies of the reproductive and immune systems of cattle. These physiological systems are especially important to the management of mature cows and their calves in cow-calf operations. The physiological processes studied included 1) the effect of monensin supplementation on postpartum folliculogenesis in mature Brahman cows and 2) the effect of sex and temperament on adaptive immune function of beef calves. The return to normal ovarian function following calving is dependent on populations of follicles during the early postpartum period; this was evaluated in mature Brahman cows 21 d following calving. Monensin supplementation provided to Brahman cows from late gestation until early lactation increased recruitment of antral follicles. Additionally, neither cow nor calf BW were affected by monensin supplementation. Because immune and stress responses are sexually dimorphic and vary amongst temperaments two experiments were conducted to determine the effects of sex and temperament on response to *Salmonella* Newport Extract vaccine at weaning in *Bos taurus* and *Bos indicus* beef calves. There was a sexually dimorphic immune response both in *Bos taurus* and *Bos indicus* calves as well as a significant influence of temperament on immune response in *Bos indicus* calves. However, the degree of immune response appeared to be adequate to confer immunity after the booster vaccination. The findings of these experiments are applicable to the management of the reproductive and immune systems of cattle thus benefiting beef cattle production.

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NOMENCLATURE

ADG	Average daily gain
AF	As-fed
BCS	Body condition score
BW	Body weight
CL	Corpus luteum
d	day
DM	Dry matter
E2	Estradiol
ELISA	Enzyme-linked immunosorbent assay
EV	Exit velocity
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
hd	Head
hr	Hour
HPO	Hypothalamic-pituitary-ovarian
IgG	Immunoglobulin G
LH	Luteinizing hormone
min	Minute
mo	Month
LPS	Lipopolysaccharide
PGF2α	Prostaglandin F2α

PPI	Postpartum interval
PS	Pen score
RIA	Radioimmunoassay
SRP	Siderophore receptor and porin protein
TS	Temperament score
VFA	Volatile fatty acid
wk	Week
yr	Year

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

In the beef cattle industry, reproductive performance is more economically important than meat and milk production (Trenkle and Willham, 1977). Determining methods to increase efficiency of reproduction and improve the fertility of beef cows can benefit producers. To maximize reproductive performance, it is imperative for a cow to produce a calf at yr intervals (Hardin and Randel, 1983). Consequently, this means that a Brahman cow must return to estrus and successfully be bred within 72 d of calving if one assumes an average gestation length of 293 d. The return to estrous occurs when the GnRH pulse generator is stimulated to promote folliculogenesis and steroidogenesis. Two primary factors that influence the length of the post-calving anestrus period include energy balance and offspring suckling (Rhodes et al., 2003).

Ionophores, such as monensin, increase ruminal propionate production (Richardson et al., 1976; McCartor et al., 1979), which leads to an increase in glucose production. A positive relationship between the increase in glucose associated with consuming monensin and reproductive performance has been reported (McCartor et al., 1979; Bushmich et al., 1980; Hixon et al., 1982; Randel et al., 1982; Hardin and Randel, 1983; Reed and Whisnant, 2001; Tallam et al., 2003). However, the connection between them is unclear. It has been reported that reproductive performance, such as the number of follicular waves (Reed and Whisnant, 2001) increased when monensin was fed to cows. Additionally, a reduction in PPI has been reported for cows fed diets containing monensin (Hardin and Randel, 1983; Sprott et al., 1988). The PPI is defined as the

period of time from calving to the first ovulation (Rhodes et al., 2003). The return to normal ovarian function following calving is dependent on populations of follicles during the early postpartum period. The goal of this review is to examine the current research pertaining to monensin supplementation and its effect on reproductive performance of cattle.

Gluconeogenesis

Understanding gluconeogenesis is critical when considering the role monensin plays in reproductive performance. Glucose is broken down in the rumen from the carbohydrates that are consumed. In the rumen, glycolysis produces pyruvate, which can then be fermented to VFAs, such as propionic, butyric, and acetic acid, as well as waste gases, such as methane and carbon dioxide. Propionic acid is formed when pyruvic acid reacts with hydrogen. Propionate is absorbed through the walls of the rumen and large intestine into the portal blood to the liver. Propionic acid becomes the principal volatile fatty acid necessary for gluconeogenesis (McCartor et al., 1979; Randel, 1990). The increased ruminal production of propionate enhances metabolic performance which maintains or slightly increases rate of gain while decreasing feed intake (Richardson et al., 1976; Randel and Rhodes, 1980; Lean et al., 1994).

Female reproduction

Hypothalamic-pituitary-ovarian axis

The integrated system that controls the production and secretion of hormones in response to the activities of the ovary is known as the hypothalamic-pituitary-ovarian (HPO) axis. The HPO axis, as demonstrated in Figure 1, is first stimulated at the

hypothalamus where GnRH is secreted from neurons. It is the stimulation of this GnRH pulse generator that promotes further endocrine actions of the HPO axis (Zieba et al., 2004; Randel and Welsh, 2013). As GnRH travels through the portal blood system to the anterior pituitary gonadotropins, LH and FSH are released (Bastidas, 1989). After this, LH and FSH travel through the vasculature of the body to the female gonad, the ovaries. Pulsatile waves of FSH stimulate the follicular waves that occur in the ovary. Following this action, the pulsatile secretions of LH stimulate dominance in the tertiary follicle being developed (Cools et al., 2014). Before ovulation, mature follicles produce estradiol which is necessary for stimulation of the GnRH surge from the hypothalamus. After a follicle has gone through this process and has not become atretic, the hypothalamic surge center stimulates secretion of GnRH to stimulate a preovulatory surge of LH, which then causes ovulation.

The size of ovarian follicles is positively correlated with estradiol concentrations (Spicer and Echternkamp, 1986). Estradiol can have a stimulatory effect on the hypothalamus, which causes the ovulatory LH surge, or an inhibitory effect on the pituitary, reducing FSH secretion (Figure 1). The described positive feedback effect is caused by rapidly increasing estradiol concentrations while low estradiol concentrations cause negative feedback (Randel et al., 1982; Hardin and Randel, 1983). During estrus in cows, estradiol increases from two- to six-fold more than estradiol concentrations characteristic of the mid-luteal phase of the estrous cycle (Spicer and Echternkamp, 1986). Once ovulation has occurred, the CL will form at the site of ovulation to produce progesterone. Progesterone acts to inhibit LH secretion and prepares the uterine

environment for pregnancy. However, if pregnancy occurs, the production of progesterone will continue in order to maintain pregnancy, and thus, stop ovarian cyclicity. If implantation of the embryo has not occurred, the uterine endometrium releases PGF2α to induce luteolysis, or CL regression. This allows for the resumption of the ovarian cycle to occur.

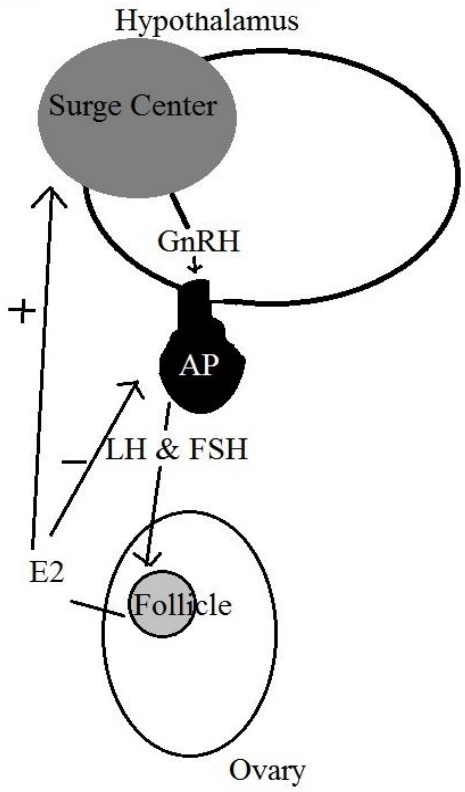

Figure 1. Hypothalamic-pituitary-ovarian (HPO) axis. AP; anterior pituitary gland. Modified from Senger, 2003.

Folliculogenesis

Folliculogenesis is the process of maturation that ovarian follicles undergo to become a candidate for ovulation. Follicles, which contain one oocyte each, go through the stages of primordial, primary, secondary, tertiary, and Graafian follicles (Ross and Schreiber, 1986). As follicles continue to mature, the cells of the follicular layer develop further (Figure 2). The stages of primordial and primary follicles occur in the female fetus (Hatzirodos et al., 2014). Lining the inside of the primary follicles are flattened granulosa cells that become part of the follicular layer (Smitz and Cortvrindt, 2002). Once the preovulatory tertiary stage has been reached, the follicular layer cells have differentiated into the granulosa layer and the two thecal layers: theca interna and theca externa. The innermost layer, the granulosa layer, contains FSH receptors that allow FSH binding which is necessary for follicular recruitment (Spicer and Echternkamp, 1986). The amount of FSH receptors does not change in the mature follicle. However, the amount of FSH bound and unbound to receptors differs (Spicer and Echternkamp, 1986). Increases in FSH concentrations coincide with the waves of follicle recruitment (Ginther et al., 1996). If a preovulatory follicle's growth has been stimulated by FSH and becomes dominant it enters the Graafian follicular stage and is now ready for ovulation.

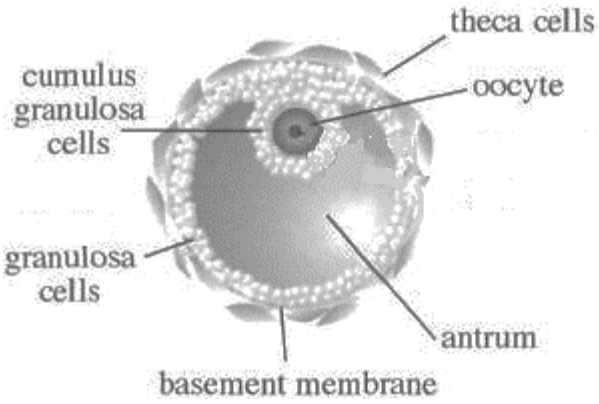

Figure 2. Structure of the ovarian follicle. Modified from Hardy et al., 2000.

In cattle, the estradiol produced by large follicles (>6 mm) originates from the granulosa cells, thus sizes of ovarian follicles are positively correlated with estradiol concentrations (Spicer and Echternkamp, 1986). The hypothalamic-hypophyseal mechanism is responsible for the estradiol-induced LH surge in prepubertal heifers (Randel et al., 1982). Due to the increase in follicular fluid estradiol, the LH surge causes the dominant follicle to ovulate. Concentrations of LH receptors increase before the ovulatory LH surge occurs and decline before ovulation.

The pool of healthy ovarian follicles, both primordial and antral, is referred to as the ovarian follicular reserve and can possibly determine the length of the reproductive lifespan of a female (Fortune et al., 2013). The ovarian follicular reserve size is positively correlated with fertility of cows (Fortune et al., 2013). Follicular development begins again approximately 7 to 15 d postpartum (Murphy et al., 1990). After

folliculogenesis is resumed, anestrus can be lengthened because of delays in ovulation of the dominant follicle (Murphy et al., 1990).

Large follicle dynamics

The follicle selected for dominance is either greater in size and is the first to develop or grows at a faster rate than the follicle that was originally larger in size (Ginther et al., 1996; Ginther, 2000). Measuring the increase in diameter of the largest and second largest follicle of each ovary is a method to determine follicular growth because follicular diameter of the largest ovarian follicles increase from ovulation of the previously dominant follicle until 20-d after that ovulation (Spicer and Echternkamp, 1986). The growth rate of oocytes coincides with the amount of granulosa cells surrounding the oocyte. This is because granulosa cells regulate development of oocytes (Fair, 2003). The primary source of ovarian steroids comes from the largest follicles. As discussed previously, these ovarian steroids inhibit gonadotrophin secretion which regulates folliculogenesis.

Rocha et al. (2015) reported that the diameter of the largest follicle increases and the PPI is shortened when BCS increases. However, the level of feeding (groups were fed 60% or 115% of NRC recommended energy requirements) in postpartum beef cows had no influence on the diameter of the largest follicle (Lishman et al., 1979). This may indicate that body condition is more influential than level of feeding on follicular growth and maturation.

Antral follicle count

Antral follicle count is the combined amount of developing follicles within both ovaries. Antral follicles are characterized by a diameter of 4 mm or greater and contain a developmentally competent oocyte. This can be counted via ultrasonography or histologically. Ireland et al. (2008) demonstrated that antral follicle count in 12 to 18 mo old Hereford X Angus X Charolais heifers was positively associated with ovarian weight and diameter and the number of healthy ovarian follicles. This determines the reproductive lifespan of the individual in that the number of times possible to conceive is increased. The number of antral follicles remain constant in beef cows up to 10 yr of age, then declines by almost 50% five to ten yr later (Spicer and Echternkamp, 1986). However, in postpartum anestrous beef cows, follicular maturation is limited (Lishman et al., 1979). This is due to the FSH requirement for antral follicle formation.

The various changes in peaks and waves of gonadotropin concentrations influence recruitment and selection of follicles, thus a large number of growing antral follicles would be necessary throughout the reproductive lifespan. The growth rate of small antral follicles to large antral follicles is positively related with the rate of atresia as the estrous cycle approaches ovulation. Atresia is the term for a follicle characterized by the deteriorating antrum of the follicle (Ross and Schreiber, 1986).

Postpartum interval

Gestation in the Brahman cow lasts approximately 293 d (Plasse et al., 1968). Consequently, a short PPI is critical to maximize the number of chances a cow has to conceive during a limited breeding season.

The length of the PPI is influenced by prepartum nutrition, postpartum nutrition, and offspring suckling. During the postpartum period, nutritional requirements increase because of lactation. By limiting suckling and improving energy balance, a lactating cow can return to estrus sooner (Mason and Randel, 1983; Del Vecchio et al., 1988; Randel, 1990). Although both of these events influence the return to estrous cyclic activity, it is more practical for most beef producers to improve energy balance of the cow than to limit calf suckling.

Reproductive performance is strongly influenced by BW and BCS change (Randel, 1990). Body condition is described as the ratio of the amount of fat to the amount of non-fatty matter in the body and is an important tool for predicting reproductive performance of cows (Smith et al., 1980; Herd and Sprott, 1996). Increasing both prepartum and postpartum energy will reduce PPI and hasten return to ovarian activity after calving (Hardin and Randel, 1983; Rhodes et al., 2003). Limiting energy intake during these periods will decrease BW, BCS, and the proportion of cows that return to estrus early in the breeding season (Whitman, 1976; Randel, 1990). Rutter and Randel (1984) observed that PPI decreases when postpartum BCS is maintained. Cows that calve at a BCS of 7 to 9, despite prepartum and postpartum BW change, returned to estrus within 60 d of calving (Whitman, 1976; Randel, 1990). When BCS increases, the PPI is shortened and the diameter of the largest follicle increases (Rocha et al., 2015).

Though improving both prepartum and postpartum energy is important to post-calving reproductive performance, it is more practical for a producer to improve energy

intake and BCS of a cow before she begins lactating. Improving prepartum energy has been reported to be more critical than improving postpartum nutrition for shortening the PPI (Wiltbank et al., 1962; Dunn and Kaltenback, 1980). Regardless of high- or low-postpartum nutrition, the PPI of suckled cows was reduced (Whitman, 1976).

Monensin

Some feed additives have been shown to improve performance of beef cattle while maintaining or decreasing the amount of feed consumed. In ruminants, this can be achieved by manipulating ruminal fermentation so that waste, such as methane, and protein degradation are decreased while improving energy availability. For example, sodium monensin is a carboxylic polyether ionophore antibiotic produced by *Streptomyces cinnamonensin* (Bergen and Bates, 1984; Bell et al., 2015). Monensin ($C_{36}H_{61}O_{11}Na$) maintains or slightly increases the rate of gain while feed intake is decreased (Goodrich et al., 1984; Schelling, 1984). The rumen microflora is altered to favor propionate production, thus increasing propionate in the rumen (Schelling, 1984). Russell and Strobel (1989) described the effects of monensin in the rumen as being characterized by a decrease of ammonia, methane, and lactic acid production while also increasing protein availability, ruminal pH, and feed digestion. Of all the VFAs produced, propionic acid is the most efficiently produced VFA for energy use within the rumen (Schelling, 1984; Randel, 1990). Butyric and acetic acid are less efficient for energy use because they require extra energy to be broken down. The percentages of efficiency for fermenting six carbon sugars (hexose) to VFAs are acetate 62%, butyrate 78%, and propionate 109% (Chalupa, 1977).

Monensin effect on cellular physiology

Ionophores, such as monensin directly affect the physiology of ruminal bacteria that produce the various VFA which consequently effects ruminant performance (Bergen and Bates, 1984). Characterized as an antiporter ion carrier, monensin specifically carries the cations Na^+ or K^+ in exchange for H^+ ions (Bergen and Bates, 1984; Bell et al., 2015). As described by Russell and Strobel, (1989) in order for bacteria to maintain a neutral pH and ion gradient, Na^+ must be maintained at a low concentration while K^+ concentration and the pH are kept elevated within the bacteria (intracellular). Outside of the bacterial membrane (extracellular), the concentration of these ions is opposite. Monensin exchanges both extracellular Na^+ for intracellular H^+, and intracellular K^+ for extracellular H^+, which eventually disrupts the intracellular pH and ion gradient equilibrium (Bell et al., 2015; Figure 3). In the cell, active transport attempts to maintain this equilibrium. In doing so, energy is depleted resulting in cell death.

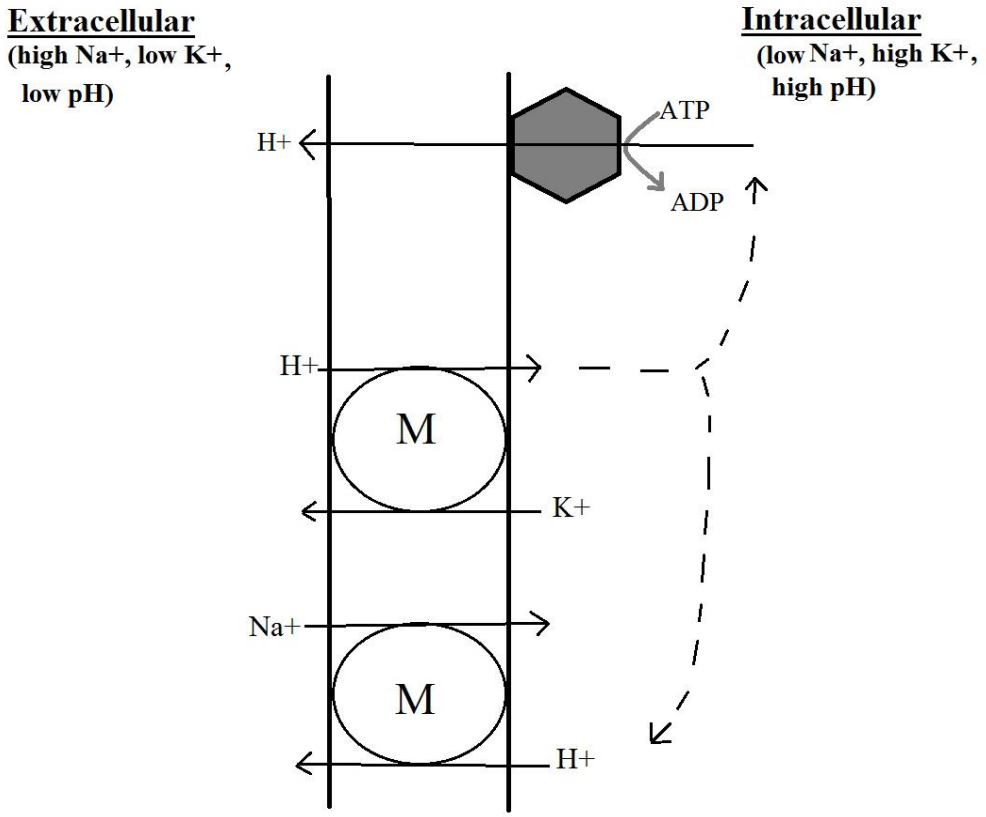

Figure 3. Mechanism of monensin (M) on gram positive ruminal bacteria. Modified with permission from Bell et al., 2015.

Two types of ruminal bacteria are gram-negative and gram-positive. Gram-negative bacteria differ from gram-positive bacteria due to their cellular structure and what VFAs and waste they produce. Gram-negative bacteria have a moderately impermeable outer membrane causing monensin to be ineffective at altering the intracellular environment (Russel and Strobel, 1989). Because gram-positive bacteria do not have this outer membrane and have a permeable peptidoglycan layer, monensin

greatly affects the cellular equilibrium discussed previously causing cell death (Bergen and Bates, 1984; Bell et al., 2015)

Monensin effects on weight gain and feed efficiency

Forage quality and stage of production control the degree of change in body weight, body condition, and feed intake of the cow in response to ionophores included in the diet (Sprott et al., 1988). However, a consistent response in weight gain or feed intake from monensin supplementation has been reported when cattle graze low quality forages (Lemenager et al., 1978; Vendramini et al., 2015). Though there was an increase in propionate concentrations, monensin supplementation did not affect ADG of Brahman crossbred heifers grazing warm-season bahiagrass (*Paspalum notatum*; Vendramini et al., 2015). In the first of a two part study, Rouquette et al. (1980) reported that monensin supplemented calves (age, 283 d) tended to have 0.10 kg higher ADG ($P < 0.10$) than controls. Each group was fed 14 % protein feed while grazing "Coastcross I" Bermudagrass. In the second part of the study, starting at 265 d of age, steer calves grazing common Bermudagrass obtained an ADG increase of 45% as compared with the control group when fed 14% protein feed plus monensin for two mo (0.68 vs. 0.47 kg, respectively; Rouquette et al., 1980).

Randel and Rouquette (1976) reported that lactating Brahman x Hereford cows supplemented with monensin consumed 12.4% less feed than lactating cows that were not fed monensin. Drylot confined heifers supplemented with monensin needed 10.9% less grain concentrate range cube to achieve similar weight gains compared with heifers that were not supplemented with monensin (Moseley et al., 1977). Goodrich et al. (1984)

summarized 228 trials of feedlot steers and heifers consuming diets with or without monensin. Those that consumed monensin had an increase of 1.6% ADG and consumed 6.4% less feed than control cattle.

Monensin effects on reproduction

Monensin supplementation, results in increased propionate production (Goodrich et al., 1984; Schelling, 1984); this increase in propionate can cause a change in multiple hormone concentrations, which may impact cow reproductive performance (Moseley et al., 1977). Changes in breeding performance that occur from ionophore inclusion depend on the quality of diet, and the age and body condition of the cow (Sprott et al., 1988; Figure 4).

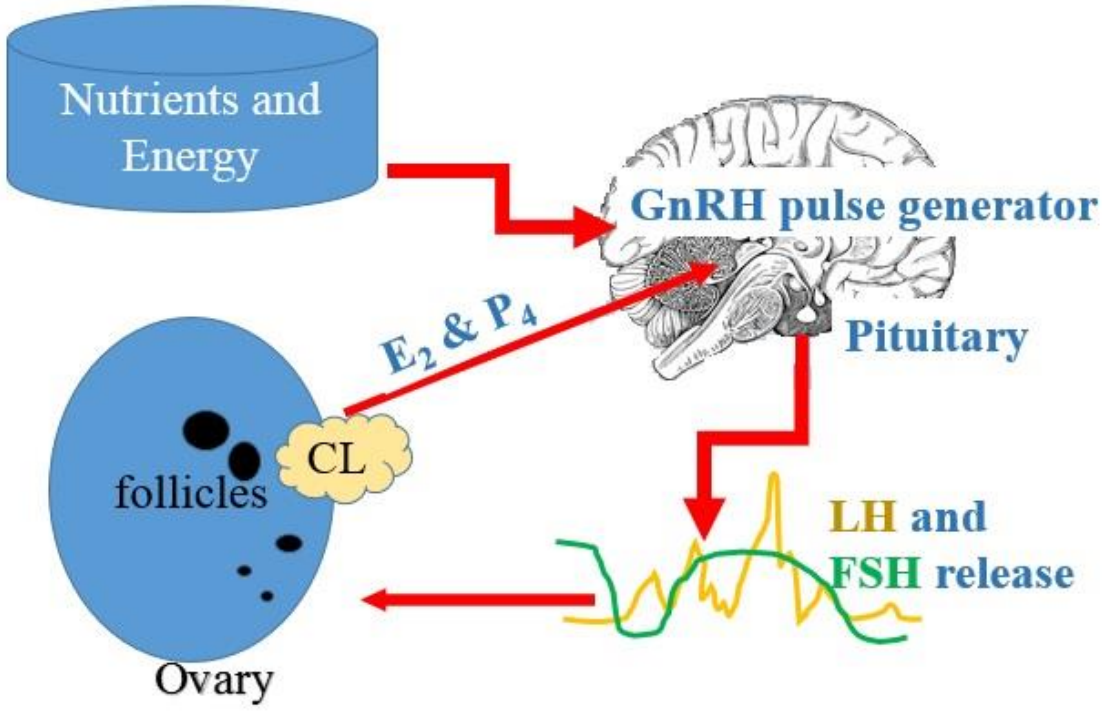

Figure 4. Effects of nutrition and energy on the HPO axis. Modified from Short and Adams, 1988.

Nutritional effects on puberty in beef heifers are under neuroendocrine control and onset of puberty is determined by the body fat stores (Randel and Welsh, 2013). Randel et al. (1982) reported that monensin, supplemented for 14 d, altered the hypothalamic-pituitary response to estradiol in prepubertal Brahman crossbred heifers. McCartor et al. (1979) reported that age at puberty was decreased by 24 d in Brangus heifers that were either supplemented with monensin or heifers that were given a high concentrate diet. Similar results were reported in which Brahman, Hereford, and

Brahman X Hereford heifers in dry lot confinement were fed grain concentrate range cubes. The heifers supplemented with monensin had a 34% increase in puberty and a 25.1% increase of propionate during the 171-d trial when compared with control heifers (Moseley et al., 1977). This confirmed the reduction in pubertal age by shifting ruminal fermentation toward higher production of propionate (Moseley et al., 1977; McCartor et al., 1979).

Sensitivity of ovarian response to exogenous gonadotropins can be improved by supplementation with monensin. Bushmich et al. (1980) reported that FSH-treated Brahman crossbred heifers fed monensin had almost twice as many corpora lutea compared with control Brahman crossbred heifers. Additionally, the monensin supplemented heifers also had an increase in ovarian weight and number of follicles. Hereford crossbred heifers supplemented with monensin consuming either a high concentrate diet (approximately 69% corn: soybean meal and 29% wheat straw: alfalfa hay) or a high forage diet (approximately 29% corn: soybean meal and 69% wheat straw) had more follicular waves than heifers not supplemented with monensin (Reed and Whisnant, 2001). Dairy cows receiving monensin (22 mg/kg) displayed no difference in dominant follicle size or antral follicle count compared with dairy cows receiving a control ration. Although, first ovulation postpartum occurred earlier for the monensin group at approximately 27 d compared with the control group at approximately 32 d (Tallam et al., 2003).

In a study (Hardin and Randel, 1983) with Brangus cows, PPI was reduced by approximately 20 d with the inclusion of monensin provided from the d after parturition

through return to estrus compared with the control group. However, this was not affected by estradiol injections (0, 1, 2, or 4 mg) 21-d postpartum for either group (Hardin and Randel, 1983). Hereford and Angus heifers received either a control or monensin supplement from 40 d prior to breeding through 120 d of lactation. This resulted in a 13.4 d decrease of PPI in monensin-supplemented heifers as compared with the control group (Hixon et al., 1982).

Monensin supplementation has the potential to reduce hay intake or decrease time for successful rebreeding (Turner et al., 1977; Lemenager et al., 1978) in beef cows. In contrast, several publications document that these production measures may remain unaltered (Randel and Rouquette, 1976; Smith et al., 1980; Tuner et al., 1980; Clanton et al., 1981). First, in a replicated study by Turner et al. (1977) pregnant, Hereford cows supplemented with monensin for 98 d prepartum (mid to late gestation) not only gained 0.2 kg more than the pregnant control cows, but hay intake decreased by 0.4 kg and PPI decreased by 8 d. Lemenager et al. (1978) also reported a tendency ($P < 0.20$) for cows supplemented with monensin to have a shortened PPI.

However, Turner et al. (1980) reported that regardless of amount of monensin (0, 50, 200, 300 mg/d for 6 mo) PPI, pregnancy rate, and calving interval were not affected; 200 mg was found to be the optimal amount of monensin to maximize feed efficiency. The first two experiments of a three part monensin supplementation study by Clanton et al. (1981) had similar results in PPI and pregnancy rate not being affected as reported by Turner et al. (1980) in regard to beef cows, although there was no difference in cow or calf weight. In the third experiment of this three part monensin supplementation study

using control and monensin (200 mg) supplemented beef heifers, there was no difference in weight gain; however, there was a numerical increase in pregnancy rate (Clanton et al., 1981). Brahman crossbred and Angus cows, with recently weaned calves, were supplemented with either a low (9 Mcal) or high (18 Mcal) energy ration with or without 125 mg of monensin (Smith et al., 1980). Cows in the high supplement group, regardless of monensin inclusion, obtained a higher pregnancy rate. Monensin supplementation did not affect pregnancy rate, although it was acknowledged that this may be due to the decrease from the recommended amount of monensin provided (125 mg vs 200 mg).

Monensin effects on calf performance

Monensin supplementation has the potential to improve calf performance (Lemenager et al., 1978; Hixon et al., 1982; Linneen et al., 2015). Angus x Hereford cross cows and heifers that were individually supplemented monensin during early lactation for 60 d raised heavier calves with greater ADG 60 d postpartum than calves from control dams (Linneen et al., 2015). Although there was no change in BW, BCS, or milk yield amongst dams from both groups. Supplementing monensin to beef cows for a longer duration during lactation affects calf BW without altering the performance of the dam. Lemenager et al. (1978) reported that calves suckling Hereford cows that were supplemented with 200 mg monensin daily for 5 mo gained 5.8 kg more than calves from control dams. Cow BW and milk yield were not affected by monensin supplementation. Supplementing monensin for approximately a mo prior to calving throughout lactation to Brahman crossbred cows did not affect calf BW at birth or through 12 wk of age (Randel and Rouquette, 1976). Additionally, treatment did not

affect cow BW or lactation although hay intake was decreased (Randel and Rouquette, 1976).

In contrast, in the Hixon et al. (1982) study of monensin supplementation provided for 40 d prior to breeding until 120 d of lactation in Hereford and Angus heifers there was a 5.9 kg increase in calf birth BW in monensin supplemented heifers as compared with the control group (Hixon et al., 1982). The final experiment of a three-part study of heifers consuming monensin also resulted in a greater calf birth weight in which calves from monensin supplemented dams weighed 30 kg as compared with 26 kg for calves from control dams (Clanton et al., 1981). Although there are studies in which monensin supplementation of dams does not alter calf performance (Randel and Rouquette, 1976; Clanton et al., 1981; Turner et al., 1980) there is some evidence that potentially improving dam feed efficiency with monensin supplementation can benefit calf performance (Lemenager et al., 1978; Hixon et al., 1982; Linneen et al., 2015).

Summary

An important benefit for cow-calf producers is having a method to increase efficiency of reproduction and improve the fertility of beef cows. Monensin increases ruminal propionate production that then increases glucose production by which an improvement of reproductive performance has been reported (McCartor et al., 1979; Bushmich et al., 1980; Hixon et al., 1982; Randel et al., 1982; Hardin and Randel, 1983; Reed and Whisnant, 2001; Tallam et al., 2003). However, the connection between the subsequent increases from monensin supplementation and reproductive performance is unclear. In order to maximize reproductive performance, it is crucial for a cow to

produce a calf at yr intervals (Hardin and Randel, 1983). Thus, the return to normal ovarian function after calving is dependent on follicular populations in the early postpartum period. Therefore, this project was designed to determine the effects of monensin supplementation from late gestation through early lactation on cow performance, follicular development 21-d postpartum, and PPI.

CHAPTER II

EFFECT OF MONENSIN SUPPLEMENTATION ON FOLLICULAR DEVELOPMENT IN POSTPARTUM BRAHMAN COWS

Synopsis

The purpose of this experiment was to evaluate the effects of monensin on follicular development in Brahman cows at 21-d postpartum. Cows which were pregnant with at least their second calf (n = 56) were stratified by age at first calving, BCS, predicted calving date, and age into two groups: control (C) cows each received 1.54 kg/d of a concentrate supplement; monensin (M) cows received the same concentrate plus 200 mg monensin/d. All cows were maintained in separate pastures with free choice access to Coastal bermudagrass hay and their allotted supplementation beginning no less than 21-d before their predicted calving dates and continued until return to estrous cyclic activity or 60 d postpartum, whichever occurred first. Ultrasonography was performed at d 21 postpartum (SonoSite M-Turbo with a 7.0 MHz L52X transducer). Each ovary was assessed for the largest follicle, second largest follicle, population of follicles 4 mm or greater in diameter, and total follicular population. Cow BW, BCS, and follicular population data were analyzed by mixed model procedures of SAS; treatment was included as a fixed effect. Follicular populations were analyzed using chi-square analysis.

Monensin tended to increase the proportion of cows with a follicular population > 10 for follicles that were 4 mm or greater in diameter (C = 17/29, 59% vs M = 21/27, 78 %; $P = 0.13$). Additionally, monensin increased the proportion of cows with a total

follicular population greater than 15 (C = 23/29, 79% vs M = 26/27, 96%; *P* = 0.05). Supplementation with monensin increased follicular populations in Brahman cows by 21-d postpartum.

Introduction

The successful return to normal reproductive function following calving is dependent on development of populations of ovarian follicles during the early postpartum period. It has been reported that reproductive performance, such as the amount of follicular waves increased when Hereford-crossed heifers were fed the ionophore, monensin (Reed and Whisnant, 2001). A positive relationship has been reported between monensin feeding and reproductive performance, which includes puberty (McCartor et al., 1979), ovarian response (Bushmich et al., 1980; Tallam et al., 2003), PPI (Hixon et al., 1982), LH response to estradiol (Randel et al., 1982; Hardin and Randel, 1983), and the number of follicular waves (Reed and Whisnant, 2001).

Therefore, it is the objective of this experiment was to determine the effects of feeding monensin from late gestation through the postpartum period on Brahman cow performance and follicular development 21-d postpartum.

Materials and methods

All experimental procedures were in compliance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching and were approved by the Texas A&M University Animal Care and Use Committee (2014-020A).

This experiment took place at the Texas A&M AgriLife Research and Extension Center, Overton, north farm located 6 miles north of Overton, TX. During the spring of

2015, 60 pregnant Brahman cows that had produced at least one calf were stratified into 2 groups based on age at first calving, BCS (determined on 1/6/2015), predicted calving date, and current age. The groups were allotted to 1 of 2 different supplements in a completely randomized design. During the experiment cows were maintained in 2 pastures, with 1 group in each pasture. Groups were fed in separate pens and were rotated between pastures at weekly intervals. Water and a complete loose mineral mix (14 to 16%, Ca; 7%, P; 12 to 14%, NaCl; 4.9%, Mg; 0.1%, K; 2,500 mg/kg, Cu; 100 mg/kg, I; 45 mg/kg, Mn; 45 mg/kg, Se; 9,900 mg/kg, Zn; 90,718.5 IU/kg, vitamin A; 9,071.8 IU/kg, vitamin D; 45.4 IU/kg, vitamin E) were provided to both groups free choice from the start of supplementation until return to estrous cyclic activity or 60 d postpartum. Because grazing was limited, Coastal Bermudagrass hay (*Cynodon dactylon*) was provided free choice during the supplementation period. Cows continuously grazed Coastal Bermudagrass pasture during the entire experiment.

Supplements (AF basis) included: 1) corn gluten feed 61.2%, cottonseed meal 35.3%, binders and lubricants 3.45% (Table 1; Control, n = 29) 2) control supplement plus 200 mg of monensin (Monensin, n = 27; Elanco, Indianapolis, IN). Supplementation began no less than 21-d before predicted calving date and continued until return to estrous cyclic activity or a maximum of 60 d postpartum, whichever occurred first. Cows expected to calve within the next 21 to 28 d began supplementation on Wednesday of each wk, starting on February 4, 2015 until April 1, 2015, at a rate of 1.54 kg/cow/d.

Cows were weighed and assigned a BCS at four specific times: (1) at the start of supplementation, (2) within 24 h of parturition, (3) upon removal from supplementation, and (4) at weaning. Body condition scores (1 = emaciated, 9 = obese) were assigned by the same evaluator throughout the experiment. A gross weight was measured and recorded for each cow within 1 h after supplementation was provided during the morning.

All calves were sired by Brahman bulls except for two calves of control dams (cows 0133 and 0152); these two calves had *Bos taurus* crossbred sires. Calving took place from March 18, 2015 until May 26, 2015. Calf BW were recorded within 24 h of birth and at weaning. Calves were weaned on October 29, 2015 (average age = 203 d; range = 156 to 235 d).

At 21-d after calving, both ovaries of each cow were evaluated for follicular development by transrectal ultrasonography (SonoSite M-Turbo with a 7.0 MHz L52X transducer; SonoSite Inc., Bothell, WA). Each ovary was assessed for the largest follicle, second largest follicle, total number of follicles that were 4 mm or greater in diameter, and total number of follicles regardless of size. Diameters were measured by the two widest points of a follicle via still image. Follicular populations were assessed via recorded video of a complete scan around each ovary. Each recorded video was viewed at a slower speed in order to accurately count follicles; this was repeated until three consecutive counts resulted in the same number of follicles. Between 8 and 10 d after the first standing estrus the ovaries were examined by ultrasound to verify CL development.

The breeding season began May 15, 2015. Cows were artificially inseminated during the first 45 d of the breeding season. Marker bulls that had been vasectomized and had penile deviation were fitted with chinball markers and used to aid in detection of standing estrus. If a cow exhibited estrus after the previous artificial insemination, the cow was artificially inseminated again. After this, the breeding season continued for 45 d with natural service by fertile bulls to comprise a 90-d total breeding season. Pregnancy and the estimated d of conception were determined at weaning by palpation per rectum of the cow's reproductive tract by one experienced evaluator.

Statistical analysis

Cow was used as the experimental unit. Continuous data were analyzed by mixed model procedures of SAS v. 9.4 (SAS Institute; Cary, NC) and the Satterthwaite approximation for degrees of freedom. Response variables included initial, final, and changes in cow BW and BCS, and calf birth and weaning weight. Models for cow data included supplementation as a fixed effect and cow age as a covariate; models for calf data included supplementation of dam and sex as fixed effects and calf sire as a random effect. Cow age was included as a covariate in calf birth and weaning BW models, and calf age was included as a covariate in calf weaning BW model. When the *P* –value for the *F* –statistic was ≤ 0.05, least squares means were separated using the LSD procedure of SAS ($\alpha = 0.05$). Follicular populations, first service conception, and pregnancy rate were analyzed using the frequency procedure of SAS and a chi-square analysis. The standard error for proportional data was calculated as $\sqrt{P (1 - P) / n}$, where P = proportion of the variable.

Four cows were removed from the experiment for the following reasons. Prior to the start of supplementation weigh in, cow 4073 (Monensin) had a full udder and was palpated as open. Cow 0152 (Control) rejected her calf following parturition. Cow 7617 (Monensin) died during a caesarian section due to a ruptured uterus. The surviving calf of cow 7617 was successfully adopted within 24 h by cow 7197 (Control) whose natural calf was found dead after parturition the following morning. Because the surviving calf of cow 7617 was successfully adopted by cow 7197, only the data from cow 7197 was used in the experiment. The calf of cow 0171 (Monensin) was found dead of unknown causes two d after parturition, thus she was removed from the experiment. Cow 2507 (Control) died of unknown causes before weaning, therefore, her weight and BCS, and her calf's BW data were not used in weaning data; however, her initial and change of BW and BCS and d 21 follicular data were included.

Results and discussion

On occasion, cows in both supplementation groups did not consume their supplement the d of and the d after parturition due to their efforts to protect and isolate their newborn calf. Of these feeding events, approximately 24% of control cows and 40% of monensin cows did not consume supplement on and/or the d after parturition.

Cow BW and BCS

As planned neither cow BW at the start of the experiment (553.6 ± 10.0 kg; P = 0.80) nor BCS at the start of the experiment (6.0 ± 0.2; P = 0.65) were different between treatments. Duration of supplementation averaged 94.5 d and was not different between treatments (P = 0.74). Additionally, neither cow BW change nor BCS change during the

prepartum supplementation period ($P > 0.65$), postpartum supplementation period ($P > 0.19$), total supplementation period ($P > 0.15$), end of supplementation to weaning ($P > 0.74$), or the entire experimental period ($P > 0.52$) were affected by supplemental treatments. Additionally, cow BW and BCS at weaning were not affected by supplementation (Table 2; $P > 0.43$). Total BW and BCS decreased over the experimental period. Most of the BW loss observed during the prepartum supplementation period is attributable to parturition; cow weights used in this calculation were taken within 24 hr after parturition.

Previous monensin supplementation studies involving *Bos taurus* cows (Lemenager et al., 1978; Linneen et al., 2015) and *Bos indicus* crossbred heifers (Bushmich et al., 1980; Vendramini et al., 2015), reported no differences in body condition and BW between cattle consuming monensin or a control diet. Although BW and BCS were not different between supplementation treatments in the current experiment, there was an unanticipated trend ($P = 0.15$) for control cows to lose less BW than cows supplemented with monensin during the supplementation period.

Calf BW performance

Supplementation of the dam did not affect calf BW at birth ($P = 0.40$) or at weaning (Table 2; $P = 0.55$). Average birth weight of calves was 36.5 kg and weaning weight average 185.4 kg, at 203 d of age. As expected, BW at birth was greater ($P = 0.02$) in males (38.0 ± 0.8 kg) than females (35.0 ± 1.0 kg) and weaning BW was greater ($P = 0.01$) in males (193.3 ± 3.9 kg) than females (177.8 ± 4.4 kg).

Similar to previous monensin supplementation studies involving *Bos indicus* crossbred (Randel and Rouquette, 1976) and *Bos taurus* cows (Clanton et al., 1981; Turner et al., 1980), calf BW at birth and weaning was not affected by the supplementation of the dam.

Follicular development

Monensin supplementation did affect antral follicular population proportions ($P < 0.06$). Figures 5 and 7 present curves derived from plotting the percentage of cows that corresponded to a given follicle population at 21-d postpartum. The greatest difference in percentages from Figure 5 are detailed in Figure 6. The percentage of cows with more than 15 follicles was greater ($P = 0.06$) for monensin supplemented (96%) cows than control supplemented cows (79%; Figure 6). In addition to total follicles the number of follicles measuring at least 4 mm in diameter is also presented (Figure 7). The greatest difference in percentages from Figure 7 are detailed in Figure 8. The percentage of cows with 10 or more antral follicles that were at least 4 mm in diameter tended ($P = 0.13$) to be greater for, monensin supplemented (78%) cows compared with control fed cows (59%; Figure 8).

The ovarian response of follicular populations to monensin supplementation in this experiment is likely because of an increase in gonadotropin receptors or an increase of gonadotropin concentrations. Similar to the results of Bushmich et al. (1980) in which monensin supplementation increased the follicular population ($P < 0.01$) of Brahman crossbred heifers that were fed pelleted concentrate and ad libitum Coastal Bermudagrass hay, Brahman cows supplemented with monensin had an increased

follicular population in this experiment. It is hypothesized that by ultrasounding ovaries at 28 d postpartum (Rocha et al., 2015) instead of 21-d postpartum, a difference in follicular development might be observed as it relates to subsequent pregnancy rates.

Pregnancy rate

Although longer than anticipated, the duration from calving to conception (average = 98.2 ± 6.0 d; $P = 0.83$; Table 2) was not affected by monensin supplementation. Additionally, inclusion of monensin did not influence pregnancy rate (58%; $P = 0.35$; Table 2). At weaning 64% of control cows and 52% of monensin cows were identified as pregnant based on palpation per rectum. Similar to the results observed in this experiment, monensin supplementation has previously been reported to not affect pregnancy rates (Turner et al., 1980; Clanton et al., 1981).

The low 58% pregnancy rate observed in this experiment was principally due to a failure of both groups to return to estrous during the breeding season. This could have been a result of the unseasonably cold and wet spring. The average annual rainfall in Overton, TX is 47.55”; however, at the Overton Center, the total rainfall for 2015 was 77.37”. During April and May 2015, the rainfall was abnormally high and the temperature was abnormally low. The average rainfall in April and May of 2015 (6.93” and 13.99”, respectively) was higher than the historic average in Overton, TX for the mo of April and May (3.6” and 4.7”, respectively; overton.tamu.edu, 2016). The average temperature in April and May of 2015 (19.2° and 22.4°C, respectively) was lower than the historic average in Overton, TX for the mo of April and May (24.4° and 28.3°C, respectively; overton.tamu.edu, 2016). The possible impact of these environmental

factors is further supported by the observation that the entire Texas A&M AgriLife Research Overton north farm herd had either delayed estrus display or none at all.

In conclusion, these data suggest that although neither cow nor calf BW were affected by monensin supplementation there was tendency for an increase in recruitment of antral follicles during the early postpartum period in cows supplemented with monensin from late gestation until early lactation.

Table 1. Nutrient composition of supplement[1] supplied daily (DM basis)	
Item	%
CP	27.90
TDN	82.34
NDF	35.13
Fat	2.00

[1]corn gluten feed 61.2%, cottonseed meal 35.3%, binders and lubricants 3.45% (AF basis)

Table 2. Effect of monensin supplementation from late gestation to display of estrous cyclic activity or d 60 postpartum on supplementation length, pregnancy rate, d to conception, and Brahman cow BW and BCS

	Supplement[1]			
	Control	Monensin	SEM[2]	*P* value[3]
No. of cows	29.0	27.0	-	-
Supplementation length, d	93.8	95.1	2.8	0.74
BW at start of supplementation, kg	555.5	551.9	10.0	0.80
BW change during prepartum supplementation period, kg	-20.6	-23.5	4.5	0.65
BW change during postpartum supplementation period, kg	-12.8	-21.9	5.1	0.20
BW change during the entire supplementation period, kg	-34.0	-46.1	6.0	0.15
BW change from end of supplementation to weaning, kg	-24.1	-21.6	5.5	0.74
BW change for entire experiment (211 to 267 d), kg	-58.5	-64.7	6.8	0.52
Weaning BW, kg	496.8	486.9	9.7	0.47
BCS at start of supplementation	6.00	5.90	0.21	0.65
BCS change during prepartum supplementation period	-0.11	-0.16	0.10	0.76
BCS change during postpartum supplementation period	-0.79	-0.88	0.16	0.69
BCS change during the entire supplementation period	-0.89	-1.02	0.15	0.58
BCS change from end of supplementation to weaning	-0.17	-0.19	0.16	0.91
BCS change for entire experiment (211 to 267 d)	-1.20	-1.22	0.16	0.90
Weaning BCS	4.86	4.68	0.16	0.43
Calf BW at birth, kg	36.0	37.0	0.9	0.40
Calf BW at weaning, kg	187.3	183.8	4.1	0.55
Days from calving to conception	97.4	99.1	6.0	0.35
Pregnancy rate at weaning, %	64.3	51.9	0.1	0.83

[1] Supplements (AF basis) included: 1) 1.54 kg/d of corn gluten feed 61.2%, cottonseed meal 35.3%, binders and lubricants 3.45% supplement (Control); 2) 200 mg/d of monensin and 1.54 kg/d of the control supplement (Monensin)
[2]Most conservative standard error used
[3]Probability of a greater F-statistic

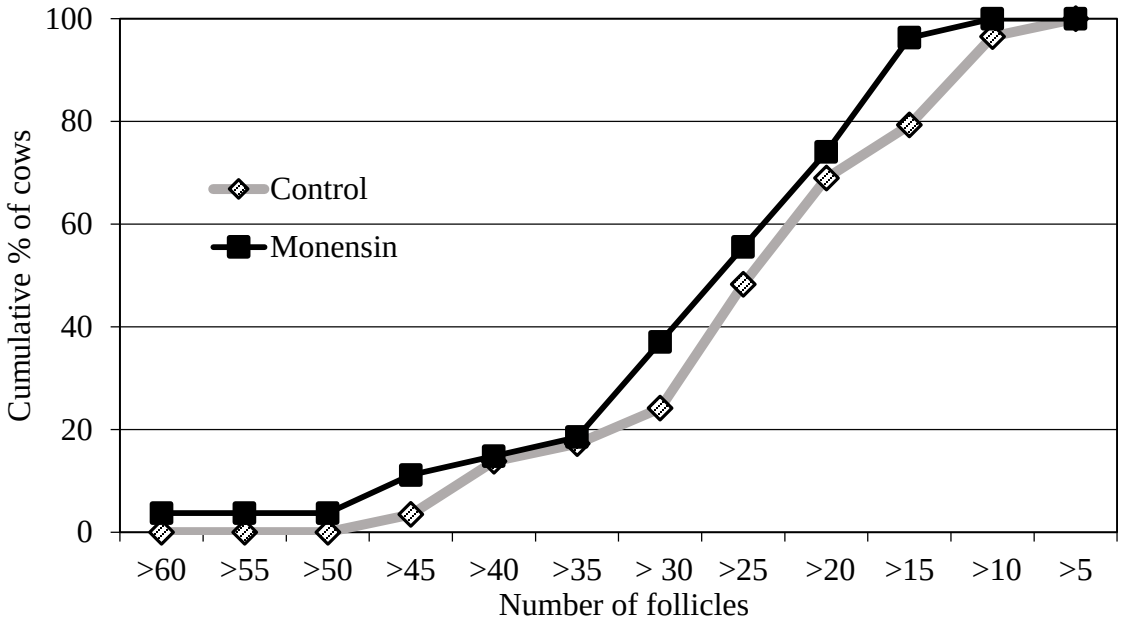

Figure 5. Cumulative percentage of Brahman cows supplemented with monensin from late gestation to early lactation with various total follicular populations at 21-d postpartum

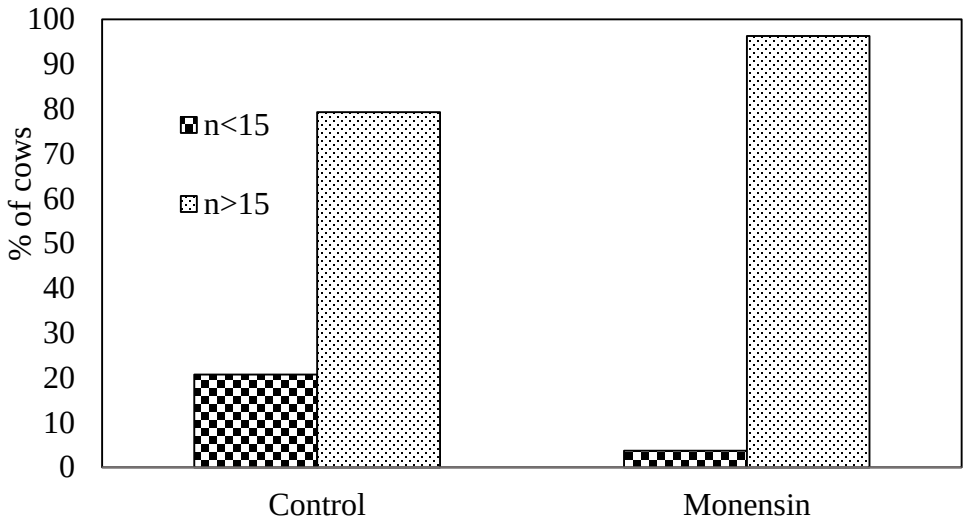

Figure 6. Percentage of Brahman cows supplemented with monensin from late gestation to early lactation with a total follicular population of > 15 follicles at 21-d postpartum (P = 0.06)

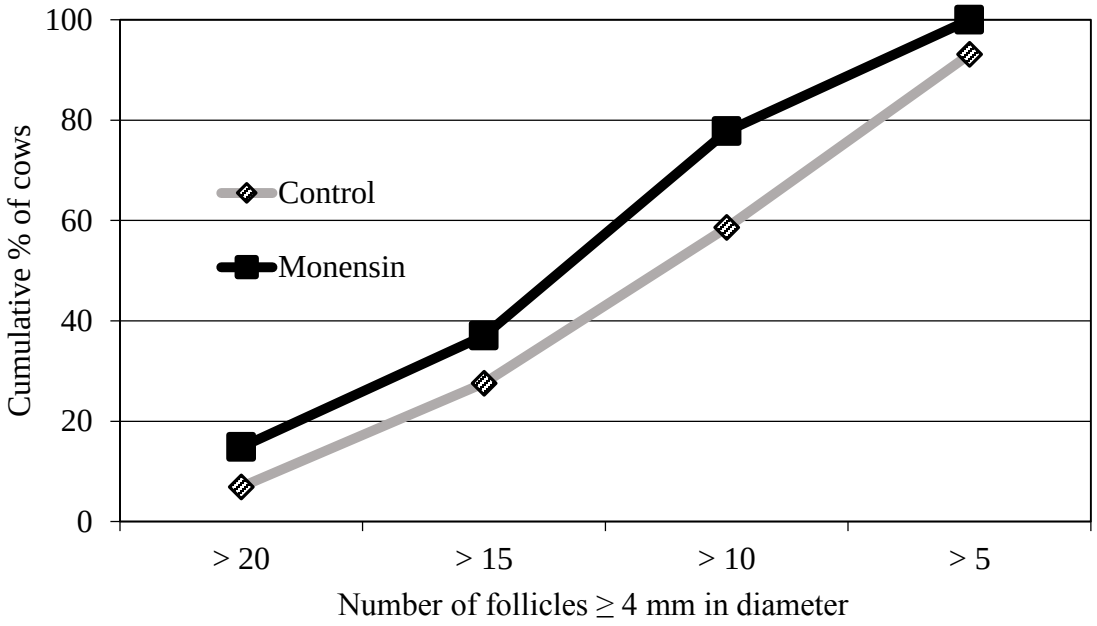

Figure 7. Cumulative percentage of Brahman cows supplemented with monensin from late gestation to early lactation with a total follicular population that consist of follicles that are a minimum of 4 mm in diameter at 21-d postpartum

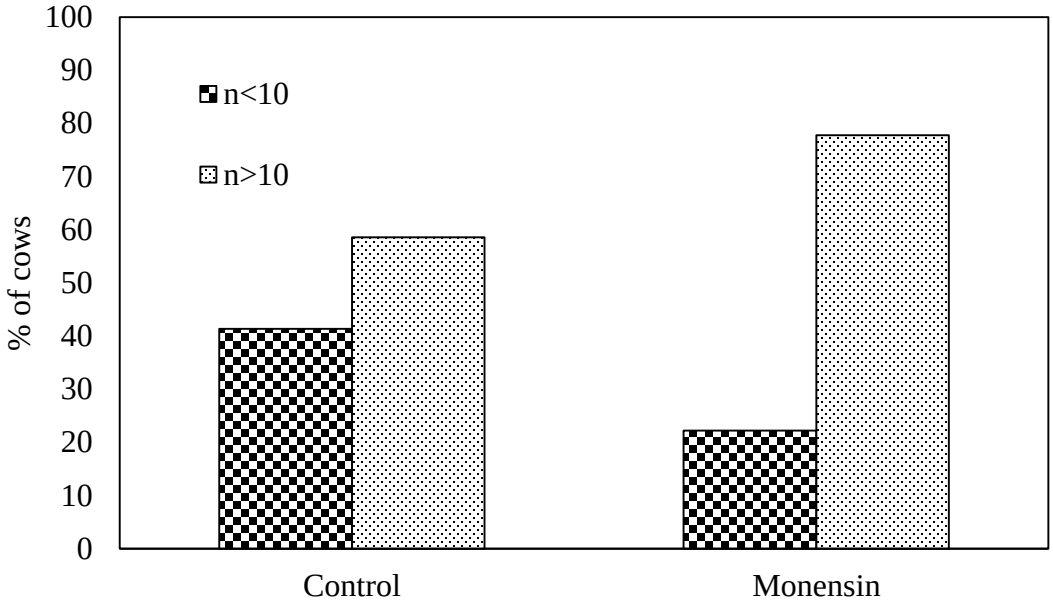

Figure 8. Percentage of Brahman cows supplemented with monensin from late gestation to early lactation with a total follicular population of > 10 follicles that are a miniumum of 4 mm in diameter at 21-d postpartum (P = 0.13)

CHAPTER III

INFLUENCE OF SEX AND TEMPERAMENT ON RESPONSE OF *BOS TAURUS* AND *BOS INDICUS* CALVES TO *SALMONELLA* NEWPORT EXTRACT VACCINE

Synopsis

The effect of sex and temperament of beef calves on the response to a commercially available *Salmonella* vaccine was studied in two experiments. Exit velocity (m/sec) and pen score (1 = calm and 5 = excitable) data collected from calves 28 d before weaning were used to assign calves to 1 of 3 temperament classes (calm, intermediate, temperamental). Calves, stratified by temperament class and sex, were assigned to groups (control or vaccinated). In Exp. 1 (n = 106) crossbred calves were assigned to non-vaccinated (control n = 54) or vaccinated (vaccinated n = 52) groups. In Exp. 2 (n = 91) Brahman calves were given an identical treatment (control n = 45; vaccinated n = 46). Vaccinated calves received 2 mL of *Salmonella* Newport Extract vaccine (Zoetis, Florham Park, NJ) twice: at weaning (d 0) and again on d 28 after weaning. Body weights (BW) were recorded on d 0, 28, and 56 after weaning and blood samples were taken at 7-d intervals from weaning until d 56 to determine serum cortisol concentrations by RIA and Ig ratios specific to the vaccine by ELISA (Epitopix, Willmar, MN). Age and BW were analyzed with mixed model procedures of SAS; sex, temperament, and treatment were included as fixed effects. As vaccination did not affect age at weaning ($P > 0.20$), BW gain from d 0 to 28 ($P > 0.33$), or from d 0 to 56 ($P > 0.40$) in either experiment only the vaccinated group was used in the remaining analysis. A specific Ig profile of each calf was assessed for peak height for both initial (Ig1; d0 to

21) and booster vaccinations (Ig2; d28 to 56) and the d these peaks occurred (referred to as Ig1d, Igd2). Neither sex ($P > 0.06$) nor temperament ($P > 0.07$) affected peak Ig1, peak Ig1d, peak Ig2, or peak Ig2d in either experiment. Cortisol and Ig ratios during the study were analyzed as repeated measures; the models included sex, temperament, and d as fixed effects. In Exp. 1 male relative to female calves had lower average cortisol (31.5 ± 2.8 vs 55.7 ± 2.8 ng/mL; $P < 0.01$) and greater average Ig ratios (0.44 ± 0.03 vs 0.34 ± 0.03; $P = 0.04$). In Exp. 2 females had greater cortisol and Ig ratios (33.11 ± 1.13 ng/mL and 0.54 ± 0.02, respectively; $P < 0.05$) and calm calves had greater Ig ratios (0.57 ± 0.02; $P < 0.05$) than other temperament classes. Overall, sex of calf influenced immune response in both experiments. However, in Exp. 2 temperament also influenced immune response to the *Salmonella* Newport Extract vaccine.

Introduction

Salmonellosis is a significant infectious zoonotic disease that affects cattle and humans. It is the cause of over 30% of bacterial foodborne deaths in humans in the United States (Mead et al., 1999; Dodd et al., 2011). Salmonellosis in both humans and other species results in enteric and septicemic infections (Snider et al., 2014). This disease affects all ages of cattle in the beef industry; however, fatalities occur most often in calves younger than 8-wk old (Smith et al., 2014; Snider et al., 2014). Specifically, the serotype of *Salmonella* Newport occurs naturally in cattle and is exceedingly virulent. The vaccine technology of the *Salmonella* Newport Extract vaccine is the siderophore receptor and porin protein (SRP) antibodies, which disrupt cell homeostasis and cause bacterial death. These antibodies bind to SRPs, which transport iron across the cell

membrane, inhibiting the iron transport necessary for maintaining cell homeostasis within the bacteria (Dodd et al., 2011).

Typical stressors for calves include weaning, castration, restraint, and transportation. These stressors can cause an increase in circulating adrenal steroid hormone concentrations, principally cortisol in cattle, which may impede growth and immune response (Burdick et al., 2011).

Temperament may affect adaptive immunity (Fell et al., 1999; Oliphint et. al., 2006; Curley et al., 2006; Burdick et al., 2011) and is defined as the reaction to stressors including handling and restraint. To measure this characteristic, calves with a higher combined pen score (Hammond et al., 1996) and exit velocity (Burrow, 1988; Curley, 2006) are described as more temperamental and thus, more likely to exhibit higher serum cortisol concentrations. Because it has been reported that temperamental animals have greater basal concentrations of hormones that are secreted due to stress and a decreased immune response to pathogens (Fell et al., 1991; Curley et al., 2008; Burdick et al., 2011), the effect of temperament on the vaccine efficacy must be investigated.

Immune and stress responses are sexually dimorphic (Hulbert et al., 2011). This means that different sexes of the same species exhibit different responses to the same stressors and immunological challenges. Carroll et al. (2015) reported sexually dimorphic differences in response to an LPS challenge in prepubertal Brahman calves. It was found that heifers had greater fever response and heart rate, though they displayed less intensive sickness behavior (display of behavior of an animal based off of a

subjective measure of 1-5) as a result of LPS induced release of pro-inflammatory cytokines than bull calves.

Effective innate and adaptive immune responses are important in host resistance to pathogens. In view of reports of sexual dimorphism and temperament variation in immune and stress responses, we conducted two experiments to determine the effects of sex and temperament on response to the *Salmonella* Newport Extract vaccine in beef calves.

Materials and methods

All experimental procedures were in compliance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching and were approved by the Texas A&M University Animal Care and Use Committee (Exp. 1, 14-028; Exp. 2, 2014-008A). These experiments were conducted from May to December of 2014. Specifically, Exp. 1 occurred from 5/20/14 until 7/15/14 and Exp. 2 occurred from 10/7/14 until 12/2/14.

Exp. 1: Influence of sex and temperament on response of Bos taurus *calves to* Salmonella *Newport Extract vaccine*

In Exp. 1 (n = 106) *Bos taurus* crossbred calves were maintained in pens with concrete bunks from weaning (d 0) to 56 d post-weaning at the Mississippi State University Brown Loam Branch Experiment Station (MAFES). The sexes included in this herd were bull (n = 28), steer (n = 26), and heifer (n = 52) calves. These sexes were combined into two sex groups; bulls and steers were classed as male calves and heifers were classed as female calves. Calves were fed approximately 1.81 kg/hd/d of 12%

crude protein pellet with water and hay free choice in different pens (approximately 25 calves/pen) separated by males and females. Pen score (1=calm and 5=excitable, PS; Hammond et al., 1996) and exit velocity (m/sec, EV; Burrow, 1988; Curley et al., 2006) data were collected from calves 28 d before weaning. Pen score is a reaction score made by one evaluator in groups of 4 to 5 calves in a confined pen. Exit velocity is the rate (m/s) a calf travels 1.83 m when exiting a squeeze chute. Results from the calculation of a temperament score (TS; Curley et al., 2006; King et al., 2006; TS = (PS + EV)/2) were used for assignment to 1 of 3 temperament classes (calm n = 31; intermediate n = 44; temperamental n = 31).

At weaning, all calves received the standard health protocol for the Brown Loam Experiment Station (Presponse, Boehringer Ingelheim, St. Joseph, MO; Pyramid 5, Boehringer Ingelheim, St. Joseph, MO; Eprinex Pour on, Merial, Duluth, Georgia). Additionally, heifers received TriVib 5L (Boehringer Ingelheim, St. Joseph, MO). Calves, stratified by temperament class and sex, were assigned to groups (control or vaccinated). Control calves (n = 54) received 2 mL of saline in front of the shoulder subcutaneously at d 0 and d 28. Vaccinated calves (n = 52) received 2 mL of *Salmonella* Newport Extract vaccine (Zoetis, Florham Park, NJ) by subcutaneous injection in front of the shoulder at d 0 (weaning) and a booster vaccination 28 d later.

Fecal samples were collected from each calf on d 0, 7, 28, and 35 to determine presence of *Salmonella*. Fecal samples (1 g) were placed into 9 ml of tetrathionate broth (BD, Sparks, MD) overnight and subsequently plated onto XLT-4 agar and MacConkey

agar plates (BD) and screened for the presence of suspect colonies. *Salmonella* fecal presence was negative in all calves.

Gross body weights were recorded on d 0, 28, and 56. Whole blood was collected into 10-mL plain red-top evacuated blood collection tubes via jugular-venipuncture at weekly intervals starting at weaning through d 56. All blood samples were centrifuged at 1700 x g x 4^{o}C x 30 min in collection tubes that had no anticoagulant in order to yield serum and then stored at -20^{o} C until all samples were obtained. All serum samples were then assayed for serum cortisol concentrations by RIA (Willard et al. 1995; Curley et al., 2010; Price et al., 2015) and IgG ratios specific to the vaccine by ELISA (Hermesch et al., 2008; Smith et al., 2014; Epitopix, Willmar, MN).

Exp. 2: Influence of sex and temperament on response of Bos indicus calves to Salmonella *Newport Extract vaccine*

In Exp. 2 (n = 91) Brahman calves were maintained in a single pasture from d 0 (weaning) to d 56 at Texas A&M AgriLife Research Overton North Farm. The calves were fed a 2:1 corn: corn gluten mix at 1% of BW daily with water and hay free choice. The sexes included in this herd were bull (males; n = 52) and heifer (female; n = 39) calves. The collection of fecal (d 7 and d 28), blood sampling (7-d intervals beginning at d 0 through d 56), and temperament data (d -28) were collected as described in Exp. 1. Temperament groups included calm (n = 29), intermediate (n = 27), and temperamental (n = 35). The calves, stratified by temperament class and sex, of Exp. 2 were given the same treatment (control, n = 45; vaccinated, n = 46) described in Exp. 1 (at d 0 and d 28). At weaning, calves received the standard health protocol for the Overton

Experiment Station (Presponse, Boehringer Ingelheim, St. Joseph, MO; Pyramid, Boehringer Ingelheim, St. Joseph, MO; Cydectin pour on, Boehringer Ingelheim, St. Joseph, MO).

Cortisol assay

Serum cortisol concentrations were evaluated from duplicate samples using a single-antibody cortisol radioimmunoassay (RIA) procedure (Willard et al., 1995; Curley et al., 2010; Price et al., 2015). Rabbit anti-cortisol antiserum (Pantex, Div. of Bio-Analysis Inc., Santa Monica, CA, Cat. #P44) was diluted 1:2,500; standard made by serial dilution (8,000 pg/100 μL to 3.9 pg/100μL) of 4-pregnen-11β,17,21-triol-3,20-dione (Steraloid Inc., Newport, RI, Cat. #Q3880–000); and radio-labeled cortisol: ^{3}H-Hydrocortisone $^{(1,2,3}$H, NEN, Boston, MA, Cat. #NET-185). Counts per min were obtained from a liquid scintillation spectrophotometric β-counter (Packard Tri-Carb 2900TR). The unknown cortisol concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK). Cortisol antiserum cross-reactivity was: corticosterone, 60%; deoxycorticosterone, 48%; progesterone, 0.01%; and estradiol, 0.01%. Interassay and intraassay CV were 11.9% and 13.3%, respectively. Data are presented as ng/mL.

IgG ratio

Laboratory staff performing ELISA testing were not aware of treatment group for any of the samples. At a concentration of 250 ng per well, with 96-wells to a microtiter plate, Salmonella Newport–derived SRP antigen was coated on plates in carbonate-coating buffer (pH, 9.6). Plates were incubated at 4°C overnight. Plates were dried and

blocked by use of 1% polyvinyl alcohol (PBS solution; 200 μL/well) to prevent nonspecific binding. Following this, plates were covered and incubated at 37°C for 2 h. Two-fold dilutions (1:100 to 1:25,600) of serum samples were set in PBS solution. These samples were then tested in duplicate. At 37°C, plates were covered and incubated for 1 hr. Plates were then washed with 0.05% PBS (Tween 20) solution 3 times. At 100 μL, horseradish-conjugated sheep anti-bovine IgG (diluted 1:1,600 in 1% sheep serum–Tween 20) was added to each well. The plates were covered and incubated at 37°C for an hr. Plates were washed with Tween 20 solution 3 times and developed by use of 100 μL of 2,2′ azino-di-3-ethyl- benzthiazoline-6-sulfonate. Optical absorbance was measured with an ELISA reader at 405 to 490 nm and sample-to-positive (S:P) ratios were calculated (Hermesch et al., 2008).

Statistical analysis

Exp. 1: Influence of sex and temperament on response of Bos taurus calves to Salmonella *Newport Extract vaccine*

Calf was used as the experimental unit. Calf BW, BW change, and age at weaning were analyzed with mixed model procedures of SAS v. 9.4 (SAS Institute; Cary, NC) and the Satterthwaite approximation for degrees of freedom. Calf sex, temperament, and treatment were included as fixed effects. Because vaccination did not affect weight gain ($P > 0.99$), only vaccinated calves were used in the remaining analysis. A specific IgG profile of each calf was assessed for maximum height for both initial (Ig1; d 0 to 21) and booster vaccination (Ig2; d 28 to 56) and the d on which these peaks occurred (referred to as Ig1d, Ig2d). IgG values were analyzed with mixed model

procedures and the Satterthwaite approximation for degrees of freedom. Fixed effects for IgG profiles were sex, temperament, and their interaction. Cortisol concentration and overall IgG ratio were analyzed as a repeated measurement with a first-order autoregressive covariance structure on the subject of calf within sex by temperament and the Kenward-Roger approximation for degrees of freedom. The models included sex, temperament, d, and their interactions as fixed effects. When the *P* –value for the *F* – statistic was ≤ 0.05, least squares means were separated using the LSD procedure of SAS (α = 0.05). Least squares means are reported in all tables and figures.

Exp. 2: Influence of sex and temperament on response of Bos indicus *calves to* Salmonella *Newport Extract vaccine*

Exp. 2 was a similar to Exp. 1 except purebred *Bos indicus* calves. After analyzing BW and age as described in to Exp. 1, vaccination did not affect BW change during the experiment (*P* > 0.88), so only vaccinated calves were used in the remaining analysis as described in Exp. 1. Least squares means are reported in all tables and figures.

Results

Exp. 1: Influence of sex and temperament on response of Bos taurus *calves to* Salmonella *Newport Extract vaccine*

Age at weaning did not differ between treatments (200 d; *P* = 0.20). Vaccination did not affect weaning BW (*P* = 0.99), weight gain from d 0 to 28 (*P* = 0.33) or d 0 to 56 (*P* = 0.43) as summarized in Table 3. As there was no difference in weight gain due to vaccination only vaccinated calves were used in the remaining analysis.

We expected BW to differ between sexes at weaning; however, there was no difference in BW at weaning between the sexes ($P = 0.35$) in the vaccinated group. No difference in BW change from d 0 to d 28 was found between the sex classifications ($P = 0.75$) or from d 0 to 56 ($P = 0.19$). Table 4 summarizes the effect of sex on BW at weaning and BW change during the experiment.

There was no difference in BW at weaning among temperament groups ($P = 0.49$). However, calm and intermediate calves tended to gain approximately 5.3 kg more over the first 28 d than temperamental calves ($P = 0.06$). From d 0 to 56 calm and intermediate tended to gain approximately 4.6 kg more than temperamental calves ($P = 0.09$). Table 5 summarizes the effect of temperament on BW at weaning and BW change during the experiment.

Neither sex (Table 6; $P > 0.19$) nor temperament (Table 7; $P > 0.07$) influenced peak Ig1 (0.34 ± 0.06), peak Ig1d (15 ± 1.5 d), peak Ig2 (0.70 ± 0.03), or peak Ig2d (41 ± 2.7 d).

From d 0 until d 56, mean cortisol concentration and IgG ratio had a relatively inverse relationship throughout the experimental period (Figure 9). As cortisol decreased, IgG ratio specific to vaccination increased over time. After each vaccination was administered, there was a large increase in IgG ratio. As displayed in Figure 10, females had higher concentrations of cortisol than males (55.7 ± 2.8 vs 31.5 ± 2.8 ng/mL; $P < 0.01$) and a lower average IgG ratio than males (0.34 ± 0.03 vs 0.44 ± 0.03; $P = 0.04$). Mean cortisol concentration (43.36 ± 3.62 ng/mL) and IgG ratio (0.39 ± 0.04) did not differ among temperaments (Figure 11; $P > 0.29$).

Exp. 2: Influence of sex and temperament on response of Bos indicus *calves to* Salmonella *Newport Extract vaccine*

Age at weaning did not differ between treatments (185 ± 3.3 d; $P = 0.35$). Vaccination did not affect weaning BW ($P = 0.76$), weight gain from d 0 to 28 ($P = 0.88$) or d 0 to 56 ($P = 0.89$). Table 8 summarizes calf BW between treatment groups. As there was no difference in weight gain due to vaccination only vaccinated calves were used in the remaining analysis.

We would expect BW to differ between sexes at weaning; however, there was no difference in BW between sexes ($P = 0.46$) in the vaccinated group. Table 9 summarizes the effect of sex on BW at weaning and BW change during the experiment. From d 0 to d 28 BW change in males and females did not differ ($P = 0.78$). However, males gained 3.9 kg more than females from d 0 to d 56 ($P = 0.02$).

There was no difference in BW at weaning among temperaments ($P = 0.71$). Table 10 summarizes the effect of temperament on BW at weaning and BW change during the experiment. Temperament groups did not differ in BW change during the first 28 d of the experiment ($P = 0.37$) nor during the entire experimental period from d 0 to d 56 ($P = 0.63$).

Neither sex (Table 11; $P > 0.06$) nor temperament (Table 12; $P > 0.33$) influenced peak Ig1 (0.57 ± 0.05), peak Ig1d (17 ± 1.04 d), peak Ig2 (0.94 ± 0.04), or peak Ig2d (46 ± 1.9 d).

The relationship between cortisol concentration and IgG ratio is summarized in Figure 12. After each vaccination was administered, there was a large increase in IgG

ratio. Female calves had higher mean serum cortisol concentrations than males (33.11 vs 25.67 ng/mL; P <0.01) and a higher IgG ratio (0.54 vs 0.48; P = 0.05) than males (Figure 13). As reported in Figure 14, temperamental calves had the greatest cortisol concentration as compared with calm calves which had the lowest cortisol concentration (36.86 ± 1.22 vs 23.47 ± 1.30 ng/mL; P < 0.01). Calm calves had greater IgG ratio (0.57 ± 0.02; P = 0.01) as compared with intermediate (0.46 ± 0.02) and temperamental calves (0.50 ± 0.02).

Discussion

The two experiments described in this report evaluated the effect of sex and temperament on the immune response of *Bos taurus* crossbred and purebred *Bos indicus* calves to the *Salmonella* Newport Extract SRP vaccine. In Exp. I, females had a greater cortisol concentration and a lower Ig ratio than males. In Exp. II, females had greater cortisol concentrations and Ig ratios than males. Additionally, in Exp. II, calm calves had lower cortisol concentrations and greater Ig ratios than other temperament classes.

As expected based on previous reports, cortisol concentrations were negatively related with adaptive immune response (Kelley, 1980; Carroll and Forsberg; 2007; Burdick et al., 2009; Burdick et al., 2011). This indicated that circulating cortisol concentrations could negatively influence the efficacy of the anti-*salmonella* vaccine which could result in an increased incidence in contracting salmonellosis or becoming a carrier. From d 0 until d 56, mean cortisol concentrations and IgG ratios had an inverse relationship throughout the Exp. 1 timeline. Although assessed in different herds in the present study, IgG increases were greater and more prolonged for purebred *Bos indicus*

calves compared with *Bos taurus* crossbred calves. This could indicate a breed variation in immune response to the *Salmonella* Newport Extract vaccine in which the IgG response of the *Bos indicus* calves was a more desirable response. Cortisol concentrations in Exp. 2 did not appear to vary as much as in Exp. 1 and did not influence the IgG ratio after vaccination.

Sexual dimorphism in adaptive and innate immune responses is evident in cattle before sexual maturation occurs (Hulbert et al., 2013; Carroll et al., 2015). Exactly how this occurs remains unclear. Females have a greater adaptive immune response than males (Nunn et al., 2009; Klein, 2012). The proposed mechanisms include differential expression of X-linked genes and microRNA, and signaling of sex steroid hormones to hormone receptors in immune cells (Klein, 2012). This dichotomy in expressions and hormone binding can differently affect responses to immunological stimuli between the sexes. A heighted stress response observed in females may be an evolutionary advantage to the offspring they are to care for (Zuk and McKean, 1996). In both experiments, females exhibited greater cortisol concentrations than males. This difference is consistent with reports of sexual dimorphism in stress response (Carroll et al., 2015). In Exp. 1 there was a negative relationship between cortisol concentration and specific IgG response in females whereas in Exp. 2 there was a positive relationship in females. Overall, sex of calf influenced immune response in both experiments.

However, in Exp. 2, temperament also influenced immune response to the *Salmonella* Newport Extract vaccine. Calm calves had the lowest stress response as reflected in cortisol concentration as compared to the other two temperamental classes.

Calm calves also had the greatest immune response in that they had the greatest IgG ratio amongst temperamental classes. Zavy et al. (1992) reported that plasma cortisol concentrations in eight-mo-old *Bos indicus* influenced steers were greater than in eight-mo-old *Bos taurus* steers. Their study indicated that breed can also influence the stress response.

In the present study, there was a sexually dimorphic response both in *Bos taurus* and *Bos indicus* prepubertal calves in both experiments as well as a significantly different temperament response in *Bos indicus* calves. However, the degree of response to the vaccine appeared adequate to confer immunity after the booster vaccination in all of the calves. Although there was a difference between sexes and amongst temperament classes, vaccinated calves reached an acceptable IgG ratio specific to vaccination that could aid in prevention of salmonellosis. Future challenge studies are necessary to confirm a differential immune response between sexes and amongst temperament classes in the presence of *Salmonella* bacteria.

Table 3. Exp. 1: Effect of *Salmonella* Newport Extract vaccine (Vaccinated) on BW of crossbred beef calves

	Vaccinated	Control	SEM[1]	*P*-value[2]
No. of calves	52	54	-	-
BW d 0 (kg)	206.3	206.3	5.7	0.99
BW change d 0 to 28 (kg)	6.6	7.8	0.9	0.33
BW change d 0 to 56 (kg)	11.7	12.9	1.1	0.43

[1] Most conservative SEM
[2] Probability of a greater *F*-statistic

Table 4. Exp. 1: Effect of sex on BW of crossbred beef calves vaccinated with *Salmonella* Newport Extract vaccine

	Female	Male	SEM[1]	*P*-value[2]
No. of calves	26	26	-	-
BW d 0 (kg)	201.1	211.6	7.9	0.35
BW change d 0 to 28 (kg)	6.3	6.9	1.4	0.75
BW change d 0 to 56 (kg)	13.0	10.4	1.4	0.19

[1] Most conservative SEM
[2] Probability of a greater *F*-statistic

Table 5. Exp. 1: Effect of temperament on BW of crossbred beef calves vaccinated with *Salmonella* Newport Extract vaccine

	Calm	Intermediate	Temperamental	SEM[1]	*P*-value
No. of calves	15	21	16	-	-
BW d 0 (kg)	213.7	198.0	207.3	10.2	0.49
BW change d 0 to 28 (kg)	8.1	8.6	3.1	1.8	0.06
BW change d 0 to 56 (kg)	14.3	12.2	8.7	1.8	0.09

a,b within a row, means without a common superscript letter differ ($P \leq 0.05$)
[1] Most conservative SEM
[2] Probability of a greater *F*-statistic

Table 6. Exp. 1: Effect of sex of crossbred beef calves vaccinated with *Salmonella* Newport Extract vaccine on attainment of peak IgG values

	Female	Male	SEM[1]	*P*-value[2]
Peak Ig1	0.30	0.39	0.05	0.22
Peak Ig1d	16	14	1	0.36
Peak Ig2	0.65	0.75	0.05	0.19
Peak Ig2d	41	41	2	0.98

d Indicates the average of the number of days elapsed until the day when the peak IgG value was attained following primary or secondary vaccination for each sex

[1] Most conservative SEM

[2] Probability of a greater *F*-statistic

Table 7. Exp. 1: Effect of temperament of crossbred beef calves vaccinated with *Salmonella* Newport Extract vaccine on attainment of peak IgG values

	Calm	Intermediate	Temperamental	SEM[1]	*P*-value[2]
Peak Ig1	0.33	0.38	0.32	0.06	0.79
Peak Ig1d	13	15	18	2	0.07
Peak Ig2	0.62	0.78	0.69	0.07	0.21
Peak Ig2d	42	40	42	3	0.76

d Indicates the average of the number of days elapsed until the day when the peak IgG value was attained following primary or secondary vaccination for each temperament class

[1] Most conservative SEM

[2] Probability of a greater *F*-statistic

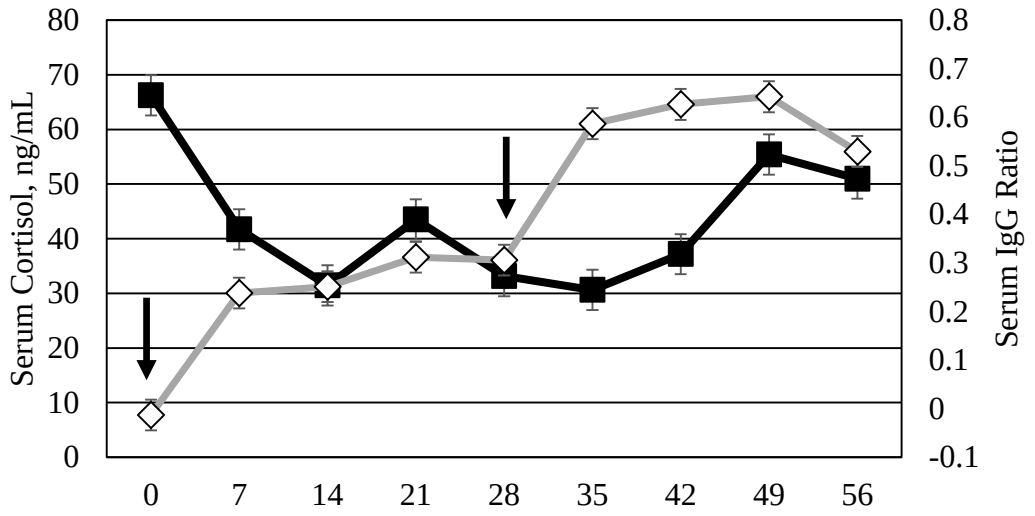

Figure 9. Exp. 1: Effect of day on mean cortisol concentration (black line) and Ig ratio (grey line) specific to *Salmonella* Newport immunization of crossbred beef calves ($P < 0.01$). Values are least squares means ± SEM. Initial vaccination occurred on d 0 and booster vaccination occurred on d 28 as indicated by the arrows.

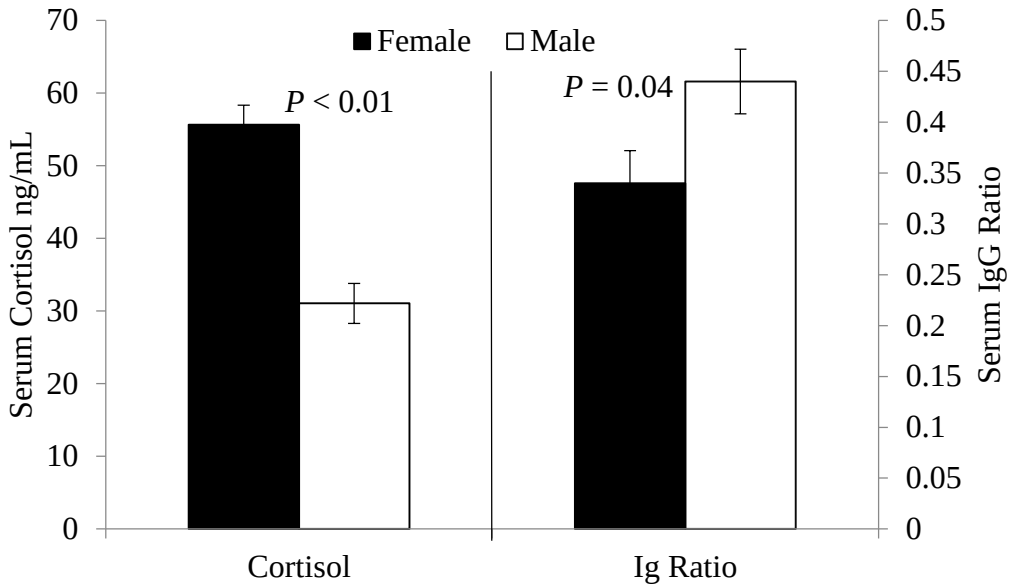

Figure 10. Exp. 1: Effect of sex on mean cortisol concentration and Ig ratio specific to *Salmonella* Newport immunization of crossbred beef calves. Values are least squares means ± SEM.

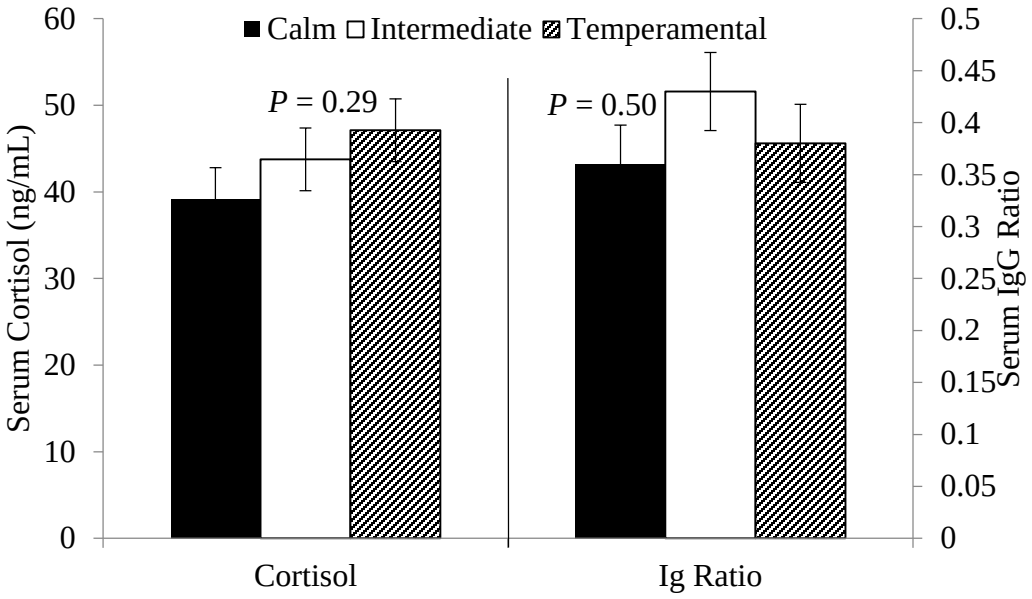

Figure 11. Exp. 1: Effect of temperament on mean cortisol concentration and Ig ratio specific to *Salmonella* Newport immunization of crossbred beef calves. Values are least squares means ± SEM.

Table 8. Exp. 2: Effect of *Salmonella* Newport Extract vaccine (Vaccinated) on BW of Brahman calves

	Vaccinated	Control	SEM[1]	*P*-value[2]
No. of calves	46	45	-	-
BW d 0 (kg)	197.2	199.0	4.1	0.76
BW change d 0 to 28 (kg)	-0.4	-0.5	0.7	0.88
BW change d 0 to 56 (kg)	7.5	7.3	1.0	0.89

[1] Most conservative SEM
[2] Probability of a greater *F*-statistic

Table 9. Exp. 2: Effect of sex on BW of Brahman calves vaccinated with *Salmonella* Newport Extract vaccine

	Female	Male	SEM[1]	*P*-value[2]
No. of calves	19	20	-	-
BW d 0 (kg)	194.2	200.3	6.3	0.46
BW change d 0 to 28 (kg)	-0.5	-0.2	0.9	0.78
BW change d 0 to 56 (kg)	5.5	9.4	1.2	0.02

[1] Most conservative SEM
[2] Probability of a greater *F*-statistic

Table 10. Exp. 2: Effect of temperament on BW of Brahman calves vaccinated with *Salmonella* Newport Extract vaccine

	Calm	Intermediate	Temperamental	SEM[1]	*P*-value[2]
No. of calves	15	14	17	-	-
BW d 0 (kg)	192.8	197.9	197.2	7.4	0.71
BW change d 0 to 28 (kg)	0.6	-0.3	-1.4	1.0	0.37
BW change d 0 to 56 (kg)	7.3	8.5	6.6	1.4	0.63

[1] Most conservative SEM
[2] Probability of a greater *F*-statistic

Table 11. Exp. 2: Effect of sex of Brahman calves vaccinated with *Salmonella* Newport Extract vaccine on attainment of peak IgG values

	Female	Male	SEM[1]	*P*-value[2]
Peak Ig1	0.62	0.52	0.04	0.06
Peak Ig1d	17	17	1	0.69
Peak Ig2	0.95	0.92	0.04	0.51
Peak Ig2d	45	47	2	0.45

d Indicates the average of the number of days elapsed until the day when the peak IgG value was attained following primary or secondary vaccination for each sex

[1] Most conservative SEM

[2] Probability of a greater *F*-statistic

Table 12. Exp. 2: Effect of temperament of Brahman calves vaccinated with *Salmonella* Newport Extract vaccine on attainment of peak IgG values

	Calm	Intermediate	Temperamental	SEM[1]	*P*-value[2]
Peak Ig1	0.62	0.52	0.56	0.05	0.36
Peak Ig1d	18	16	16	1	0.33
Peak Ig2	0.98	0.89	0.94	0.04	0.33
Peak Ig2d	44	47	46	2	0.43

d Indicates the average of the number of days elapsed until the day when the peak IgG value was attained following primary or secondary vaccination for each temperament class

[1] Most conservative SEM

[2] Probability of a greater *F*-statistic

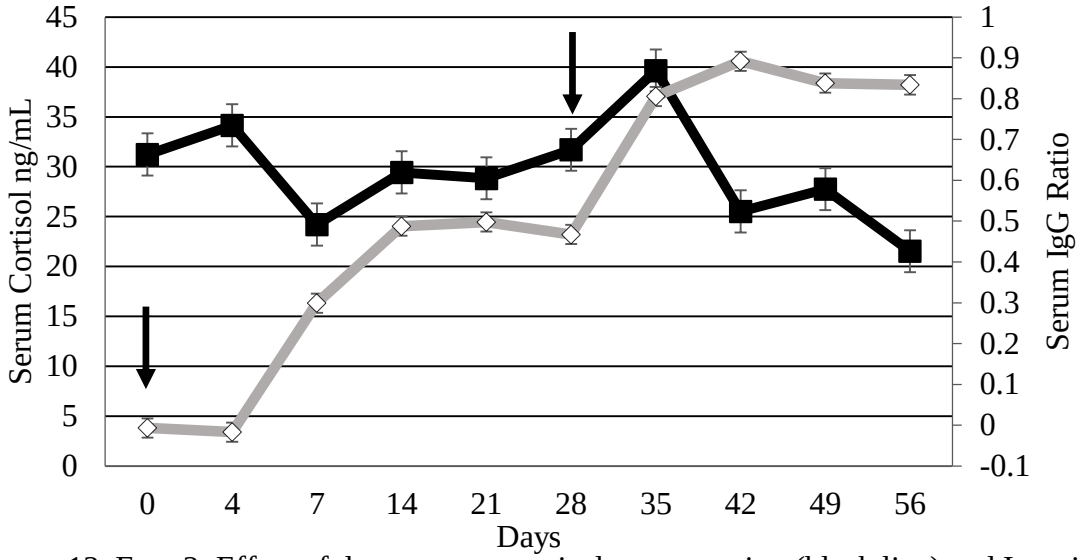

Figure 12. Exp. 2: Effect of day on mean cortisol concentration (black line) and Ig ratio (grey line) specific to *Salmonella* Newport immunization of Brahman calves ($P < 0.01$). Values are least squares means ± SEM. Initial vaccination occurred on d 0 and booster vaccination occurred on d 28 as indicated by the arrows.

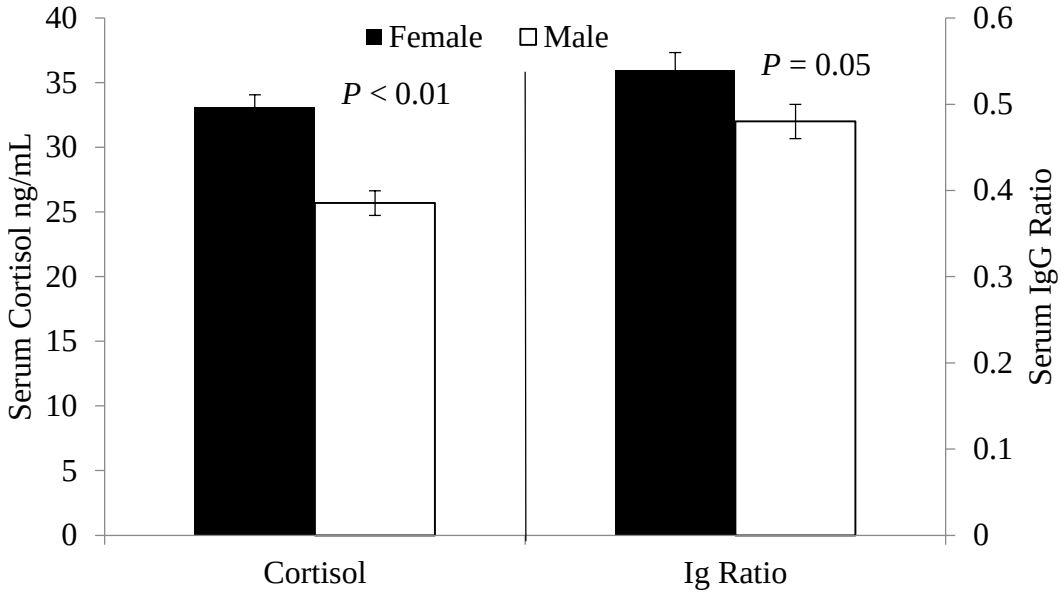

Figure 13. Exp. 2: Effect of sex on mean cortisol concentration and Ig ratio specific to *Salmonella* Newport immunization of Brahman calves. Values are least squares means ± SEM.

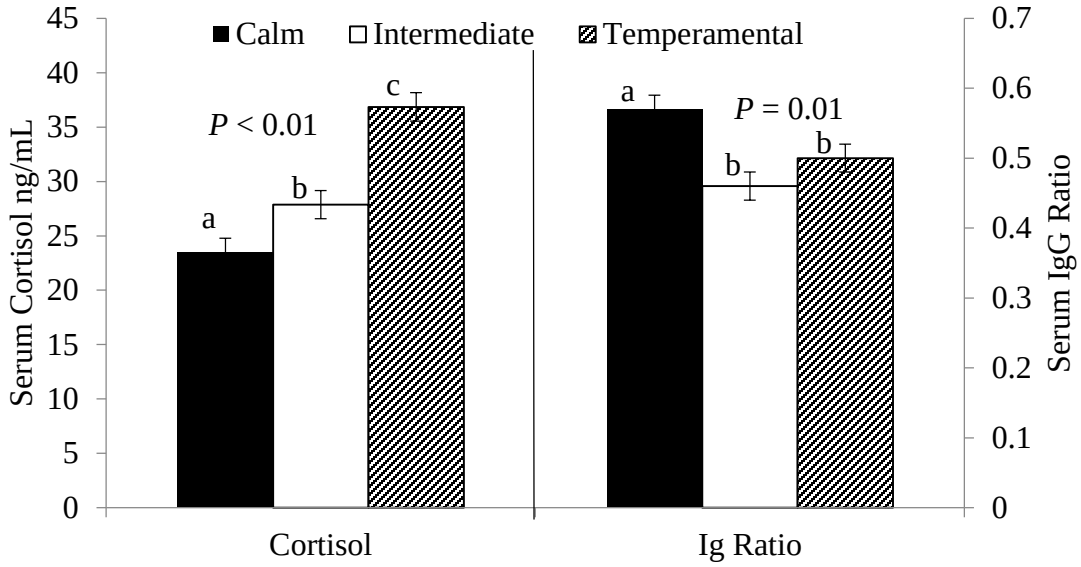

Figure 14. Exp. 2: Effect of temperament on mean cortisol concentration and Ig ratio specific to *Salmonella* Newport immunization of Brahman calves. Values are least squares means ± SEM.

CHAPTER IV

CONCLUSION

The physiologically regulated factors that can influence the cow-calf industry are reproductive longevity and stress and immune responses. Specifically, the factors studied included 1) the effect of monensin supplementation on postpartum folliculogenesis in mature Brahman cows and 2) the effect of sex and temperament on adaptive immune function of beef calves.

The return to normal ovarian function following calving is dependent on populations of follicles during the early postpartum period. An experiment was conducted to study whether use of an ionophore that preferentially enhances the VFA profile (i.e., improves energy available to the cow) would improve postpartum folliculogenesis in cows. Mature Brahman cows supplemented with monensin from late gestation through early lactation increased recruitment of antral follicles observed 21-d following calving. Monensin supplementation increased the proportion of cows with total follicular population > 15 and total follicular population > 10 that contain follicles 4 mm or wider in diameter than control cows. Additionally, neither cow nor calf BW were affected by monensin supplementation.

Two experiments were conducted to determine the effects of sex and temperament on the response to *Salmonella* Newport Extract vaccine at weaning in *Bos indicus* and *Bos taurus* crossbred beef calves because the various temperaments and sexes of calves can influence the immune and stress responses of an animal. There was a sexually dimorphic immune response both in *Bos taurus* crossbred and *Bos indicus*

calves. In *Bos taurus* crossbred calves females had greater cortisol but lower Ig ratios than males. However, in *Bos indicus* calves, females had greater cortisol and Ig ratios than males. Although there was no statistically significant effect of temperament on stress and immune response in *Bos taurus* calves, there was a significant influence of temperament on immune and stress response in *Bos indicus* calves. Calm *Bos indicus* calves had the lowest cortisol concentration and the greatest Ig ratios relative to the two other temperament classes. Although there was a difference between sexes and among temperament classes, all vaccinated calves maintained an acceptable IgG ratio specific to vaccination that could aid in prevention of salmonellosis.

The physiological systems of reproduction and immunity are important to the management of mature cows and their calves in cow-calf operations. The findings of these experiments are applicable to the production and management of beef cattle.

LITERATURE CITED

Bastidas, P. S. 1989. Ovarian and pituitary function during the post-partum interval in Brahman and Brahman crossbred cows. Ph.D. Dissertation. Texas A&M Univ., College Station.

Bell, N., T. Wickersham, V. Sharma, and T. Callaway. 2015. Ionophores: a tool for improving ruminant production and reducing environmental impact. In: P. K. Malik et al., editor, Livestock production and climate change. CABI, London, UK. p. 263-272.

Bergen, W. G. and D. B. Bates. 1984. Ionophores: their effect on production efficiency and mode of action. J. Anim. Sci. 58:1465–1483.

Burdick, N. C., J. P. Banta, D. A. Neuendorff, J. C. White, R. C. Vann, J. C. Laurenz, T. H. Welsh, Jr., and R D. Randel. 2009. Interrelationships among growth, endocrine, immune, and temperament variables in neonatal Brahman calves. J. Anim. Sci. 87:3202-3210.

Burdick, N. C., R. D. Randel, J. A. Carroll, and T. H. Welsh, Jr. 2011. Interactions between temperament, stress, and immune function in cattle. International J. Zool. doi:10.1155/2011/373197.

Burrow, H. M., G. W. Seifert, and N. J. Corbet. 1988. A new technique for measuring temperament in cattle. Proc. Aust. Soc. Anim. Prod. 17:154-175.

Bushmich, S. L., R. D. Randel, M. M. McCartor, and L. H. Carroll. 1980. Effect of dietary monensin on ovarian response following gonadotropin treatment in prepuberal heifers. J. Anim. Sci. 51:692–697.

Carroll, J. A. and N. E. Forsberg. 2007. Influence of stress and nutrition on cattle immunity. Vet. Clin. N. Am.: Food Animal Practice. 23:105–149.

Carroll, J.A., N.H. McArthur, and T.H. Welsh, Jr. 2007. In vitro and in vivo temporal aspects of ACTH secretion: stimulatory actions of corticotrophin-releasing hormone and vasopressin in cattle. J. Vet. Med. A 54:7-14.

Carroll, J. A., N. C. Burdick Sanchez, L. E. Hulbert, M. A. Ballou, J. W. Dailey, L. C. Caldwell, R. C. Vann, T. H. Welsh, Jr., and R. D. Randel. 2015. Sexually dimorphic innate immunological responses of pre-pubertal Brahman cattle following an intravenous lipopolysaccharide challenge. Vet. Immun. and Immunop. 166:108–115.

Chalupa, W. 1977. Manipulating rumen fermentation. J. Anim. Sci. 45:585–599.

Clanton, D. C., M. E. England, and J. C. Parrott III. 1981. Effect of Monensin on efficiency of production in beef cows. J. Anim. Sci. 53:873-880.

Cools, S., W. Van den Broeck, P. Bossaert, M. Hostens, and G. Opsomer. 2014. A field study to unravel factors that are significantly associated with the secretory activity of the corpus luteum during the first three postpartum cycles in high yielding dairy cows, based on the amount of steroidogenic and endothelial cells present in the luteal tissue. Reprod. Domest. Anim. 49:881–893.

Curley, K. O., J. C. Paschal, T. H. Welsh, Jr., and R. D. Randel. 2006. Technical note: exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J. Anim. Sci. 84:3100-3103.

Curley, K. O., D. A. Neuendorff, A. W. Lewis, J. J. Cleere, T. H. Welsh, Jr., and R. D. Randel. 2008. Functional characteristics of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. Horm. Behav. 53:20-27.

Curley, K. O., D. A. Neuendorff, A. W. Lewis, F. M. Rouquette, R. D. Randel, and T. H. Welsh, Jr. 2010. The effectiveness of vasopressin as an ACTH secretagogue in cattle differs with temperament. Physiol. Behav. 101:699–704.

Del Vecchio, R. P., R. D. Randel, D. A. Neuendorff, and L. A. Peterson. 1988. Effect of alfaprostol, lasalocid, and once-daily suckling on postpartum interval in Brahman and Brahman crossbred cattle. Theriogenology. 30:797–809.

Dodd, C. C., D. G. Renter, D. U. Thomson, and T. G. Nagaraja. 2011. Evaluation of the effects of a commercially available *Salmonella* Newport siderophore receptor and porin protein vaccine on fecal shedding of *Salmonella* bacteria and health and performance of feedlot cattle. Am. J. Vet. Res. 72:239–247.

Dunn, T.G. and C.C. Kaltenback. 1980. Nutrition and the postpartum interval of the ewe, sow, and cow. J. Anim. Sci. 51:29.

Evans, R. D. and L. C. Heather. 2016. Metabolic pathways and abnormalities. Surgery. http://dx.doi.org/10.1016/ j.mpsur.2016.03.010

Fair, T. 2003. Follicular oocyte growth and acquisition of developmental competence. Anim. Reprod. Sci. 78:203–216.

Fell, L. R., I. G. Colditz, K. H. Walker, and D. L.Watson. 1991. Associations between temperament, performance and immune function in cattle entering a commercial feedlot. Aust. J. Exp. Agr. 39:795–802.

Fortune, J. E., M. Y. Yang, J. J. Allen, and S. L. Herrick. 2013. Triennial reproduction symposium: The ovarian follicular reserve in cattle: What regulates its formation and size? J. Anim. Sci. 91:3041–3050.

Ginther, O. J., M. C. Wiltbank, P. M. Fricke, J. R. Gibbons, and K. Kot. 1996. Selection of the dominant follicle in cattle. Biol. Reprod. 55:1187–1194.

Ginther, O. J. 2000. Selection of the dominant follicle in cattle and horses. Anim. Reprod. Sci. 60:61–79.

Goodrich, R. D., J. E. Garrett, D. R. Gast, M. A. Kirick, D. A. Larson, and J. C. Meiske. 1984. Influence of monensin on the performance of cattle. J. Anim. Sci. 58:1484–1498.

Hammond, A. C., T. A. Olson, C. C. Chase, Jr., E. J. Bowers, R. D. Randel, C. N. Murphy, D. W. Vogt, and A. Tewolde. 1996. Heat tolerance in two tropically adapted *Bos taurus* breed, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. J. Anim. Sci. 74:295-303.

Hardin, D. R. and R. D. Randel. 1983. Effect of monensin on postpartum interval to first estrus and serum LH response to 0, 1, 2 or 4 mg estradiol-17β at 21 d postpartum. Theriogenology. 19:343–354.

Hardy K., C. S. Wright, S. Franks, and R. M. L. Winston. 2000. In vitro maturation of oocytes. Brit. Med. Bull. 56:588-602.

Hatzirodos, N., H. F. Irving-Rodgers, K. Hummitzsch, M. L. Harland, S. E. Morris, and R. J. Rodgers. 2014. Transcriptome profiling of granulosa cells of bovine ovarian follicles during growth from small to large antral sizes. BMC Genomics. 15:24.

Herd, D. B. and L. R. Sprott. 1996. Body condition, nutrition and reproduction of beef cows. Texas Agric. Ext. Serv. Bull. 1526, College Station.

Hermesch, D. R., D. U. Thomson, G. H. Loneragan, D. R. Renter, and B. J. White. 2008. Effects of a commercially available vaccine against *Salmonella* enterica serotype Newport on milk production, somatic cell count, and shedding of *Salmonella* organisms in female dairy cattle with no clinical signs of salmonellosis. Am. J. Vet. Res. 69:1229–1234.

Hixon, D. L., G. C. Fahey, Jr., D. J. Kesler, and A. L. Neumann. 1982. Effects of creep feeding and monensin on reproductive performance and lactation of beef heifers. J. Anim. Sci. 55:467-474.

Hulbert, L. E., J. A. Carroll, N. C. Burdick, R. D. Randel, M. S. Brown, and M. A. Ballou. 2011. Innate immune responses of temperamental and calm cattle after transportation. Vet. Immunol. Immunop. 143:66–74.

Hulbert, L. E., J. A. Carroll, M. A. Ballou, N. C. Burdick, J. W. Dailey, L. C. Caldwell, A. N. Loyd, R. C. Vann, T. H. Welsh, Jr., and R. D. Randel. 2013. Sexually dimorphic stress and pro-inflammatory cytokine responses to an intravenous corticotropin-releasing hormone challenge of Brahman cattle following transportation. Innate Immun. 19:378-387.

Ireland, J. L. H., D. Scheetz, F. Jimenez-Krassel, A. P. N. Themmen, F. Ward, P. Lonergan, G. W. Smith, G. I. Perez, A. C. O. Evans, and J. J. Ireland. 2008. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. Biol. Reprod. 79:1219–1225.

Kelley, K.W. 1980. Stress and immune function: a bibliographic review. Ann. Rech. Vet. 11:445-478.

King, D. A., C. E. Schuehle Pfeiffer, R. D. Randel, T. H. Welsh, Jr., R. A. Oliphint, B. E. Baird, K. O. Curley, R. C. Vann, D. S. Hale, and J. W. Savell. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Sci. 74:546-556.

Klein, S. L. 2012. Innune cells have sex and so should jornal articles. Endocrinology. 153:2544-2550.

Lean, I. J., M. Curtis, R. Dyson, and B. Lowe. 1994. Effects of sodium monensin on reproductive performance of dairy cattle. I. effects on conception rates, calving-to-conception intervals, calving-to-heat and milk production in dairy cows. Aust. Vet. J. 71:273–277.

Lemenager, R. P., F. N. Owens, K. S. Lusby, and R. Totusek. 1978. Monensin, forage intake and lactation of range beef cows. J. Anim. Sci. 47:247–254.

Linneen, S. K., A. L. McGee, J. R. Cole, J. S. Jennings, D. R. Stein, G. W. Horn, and D. L. Lalman. 2015. Supplementation of monensin and Optimase to beef cows consuming low-quality forage during late gestation and early lactation. J. Anim. Sci. 93:3076–3083.

Lishman, A. W., S. M. J. Allison, R. L. Fogwell, R. L. Butcher, and E. K. Inskeep. 1979. Follicular development and function of induced corpora lutea in underfed postpartum anestrous beef cows. J. Anim. Sci. 48:867–875.

Mason, G. L. and R. D. Randel. 1983. Effect of monensin and suckling on the GnRH induced luteinizing hormone surge and the effect of monensin on the postpartum interval in Brangus cows. Theriogenology. 19:331–342.

McCartor, M. M., R. D. Randel, and L. H. Carroll. 1979. Dietary alteration of ruminal fermentation on efficiency of growth and onset of puberty in Brangus heifers. J. Anim. Sci. 48:488–494.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerging Infect. Dis. 5:607–625.

Moseley, W. M., M. M. McCartor, and R. D. Randel. 1977. Effect of monensin on growth and reproductive performance of beef heifers. J. Anim. Sci. 45:961-968.

Murphy, M. G., M. P. Boland, and J. F. Roche. 1990. Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. J. Reprod. Fertil. 90:523–533.

Nunn, C. L., P. Lindenfors, E. R. Pursall, and J. Rolff. 2009. On sexual dimorphism in immune function. Phil. Trans. R. Soc. B. 364:61–69.

Oliphint, R. A. 2006. Evaluation of the inter-relationships of temperament, stress responsiveness and immune function in beef calves. M.S. Thesis. Texas A&M Univ., College Station.

Plasse, D., A. C. Warnick, R. E. Reese, and M. Koger. 1968. Reproductive behavior of Bos indicus females in a subtropical environment. II. gestation length in Brahman cattle. J. Anim. Sci. 27:101–104.

Price, D. M., A.W. Lewis, D. A. Neuendorff, J. A. Carroll, N. C. Burdick Sanchez, R. C. Vann, T. H. Welsh, Jr., and R. D. Randel. 2015. Physiological and metabolic responses of gestating Brahman cows to repeated transportation. J. Anim. Sci. 93:737-745.

Randel, R. D. and F. M. Rouquette, Jr. 1976. Effect of monensin on lactation in beef cows. J Anim. Sci. 43:232. (Abstr.)

Randel, R. D. and R. C. Rhodes III. 1980. The effect of dietary monensin on the luteinizing hormone response of prepuberal heifers given a multiple gonadotropin-releasing hormone challenge. J. Anim. Sci. 51:925–931.

Randel, R. D., L. M. Rutter, and R. C. Rhodes III. 1982. Effect of monensin on the estrogen-induced LH surge in prepuberal heifers. J. Anim. Sci. 54:806–810.

Randel, R. D. 1990. Nutrition and postpartum rebreeding in cattle. J. Anim. Sci. 68:853–862.

Randel, R. D. and T. H. Welsh. 2013. Joint alpharma-beef species symposium: interactions of feed efficiency with beef heifer reproductive development. J. Anim. Sci. 91:1323–1328.

Reed, B. K. and C. S. Whisnant. 2001. Effects of monensin and forage: concentrate ratio on feed intake, endocrine, and ovarian function in beef heifers. Anim. Reprod. Sci. 67:171–180.

Rhodes, F. M., S. McDougall, C. R. Burke, G. A. Verkerk, and K. L. Macmillan. 2003. Invited review: treatment of cows with an extended postpartum anestrous interval. J. Dairy Sci. 86:1876–1894.

Richardson, L. F., A. P. Raun, E. L. Potter, C. O. Cooley, and R. P. Rathmacher. 1976. Effect of monensin on rumen fermentation in vitro and in vivo. J. Anim. Sci. 43:657–664.

Rocha, T.C., R. A. d'Orey Branco, R. C. Vann, T. H. Welsh, Jr., D. A. Neuendorff, A. W. Lewis, and R. D. Randel. 2015. Relationship between body condition score, follicular activity and postpartum interval in Brahman females. ASAS. Southern Section Meeting Proceedings. 49 #121. (Abstr.)

Ross, G. T. and J. R. Schreiber. 1986. The ovary. In: S. Boyd, editor, Reproductive endocrinology second edition. W. B. Saunders Co., Philadelphia, PA. p. 115-139.

Rouquette, Jr., F. M., J. L. Griffin, R. D. Randel, and L. H. Carroll. 1980. Effect of monensin on gain and forage utilization by calves grazing bermudagrass. J. Anim. Sci. 51:521-252.

Russell, J. B. and H. J. Strobel. 1989. Effect of ionophores on ruminal fermentation. Appl. Environ. Microbiol. 55:1–6.

Rutter, L. M. and R. D. Randel. 1984. Postpartum nutrient intake and body condition: effect on pituitary function and onset of estrus in beef cattle. J. Anim. Sci. 58:265–274.

Schelling, G. T. 1984. Monensin mode of action in the rumen. J. Anim. Sci. 58:1518–1527.

Senger, P. L. 1997. Pathways to pregnancy and parturition. Current Conceptions, Inc. Pullman, WA.

Short, R. E. and D. C. Adams. 1988. Nutritional and hormonal interrelationships in beef cattle reproduction. Can. J. Anim. Sci. 68:29–39.

Smith, G. W., M. L. Alley, D. M. Foster, F. Smith, and B. W. Wileman. 2014. Passive immunity stimulated by vaccination of dry cows with a *Salmonella* bacterial extract. J. Vet. Intern. Med. 28:1602–1605.

Smith, M. F., L. D. Shipp, W. N. Songster, J. N. Wiltbank, and L. H. Carroll. 1980. Effect of monensin, energy level, and cow body condition on pregnancy rate in nonlactating beef cows. Theriogenology. 14:91-95.

Smitz, J. E. and R. G. Cortvrindt. 2002. The earliest stages of folliculogenesis in vitro. Reproduction. 123:185–202.

Snider, T. A., T. Gull, T. A. Jackson, F. J. Martinez-Becerra, D. R. Picking, W. D. Picking, and W. L. Picking. 2014. Experimental salmonellosis challenge model in older calves. Vet. Microbiol. 170:65–72.

Spicer, L. J. and S. E. Echternkamp. 1986. Ovarian follicular growth, function, and turnover in cattle: a review. J. Anim. Sci. 62:428-451

Sprott, L. R., T. B. Goehring, J. R. Beverly, and L. R. Corah. 1988. Effects of ionophores on cow herd production: a review. J. Anim. Sci. 66:1340–1346.

Tallam, S. K., T. F. Duffield, K. E. Leslie, R. Bagg, P. Dick, G. Vessie, and J. S. Walton. 2003. Ovarian follicular activity in lactating Holstein cows supplemented with monensin. J. Dairy Sci. 86:3498–3507.

Trenkle, A. and R. L. Willham. 1977. Beef production efficiency. Science. 198:1009–1015.

Turner, H. A., R. J. Raleigh, and D. C. Young. 1977. Effect of monensin on feed efficiency for maintaining gestating mature cows wintered on meadow hay. J. Anim. Sci. 44:338–342.

Turner, H. A., D. C. Young, R. J. Raleigh, and D. Zo Bell. 1980. Effect of various levels of monensin on efficiency and production of beef cows. J. Anim. Sci. 50:385–390.

Vendramini, J. M. B., J. M. D. Sanchez, R. F. Cooke, A. D. Aguiar, P. Moriel, W. L. da Silva, O. F. R. Cunha, P. D. S. Ferreira, and A. C. Pereira. 2015. Stocking rate and monensin supplemental level effects on growth performance of beef cattle consuming warm-season grasses. J. Anim. Sci. 93:3682-3689.

Whitman, R. W. 1976. Weight change, body condition, and beef cow reproduction. Ph.D. Dissertation. Colorado State Univ., Fort Collins.

Willard, S. T., J. A. Carroll, R. D. Randel, P. G. Harms, and T. H. Welsh, Jr. 1995. Regulation of adrenocorticotropin secretion in vitro by anterior pituitary corticotrophs from fallow deer (*Dama dama*). Domest. Anim. Endocrinol. 12:283–292.

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, K. E. Gregory, and R. M. Koch. 1962. Effect of energy level on reproductive phenomena of mature Hereford cows. J. Anim. Sci. 21:219-225.

Zavy, M.T., P.E. Juniewicz, W.A. Phillips, and D.L. VonTungeln. 1992. Effect of initial restraint, weaning, and transport stress on baseline and ACTH-stimulated cortisol responses in beef calves of different genotypes. Am. J. Vet. Res. 53:551-557.

Zieba, D. A. 2004. Regulatory roles of leptin at the hypothalamic-hypophyseal axis before and after sexual maturation in cattle. Biol. Reprod. 71:804–812.

Zuk, M. and K. A. McKean. 1996. Sex differences in parasite infections: patterns and processes. Int. J. Parasitol. 26:1009-1024.

APPENDIX A

CORTISOL RIA

Reference:

Carroll, J.A., N.H. McArthur, and T.H. Welsh, Jr. 2007. In vitro and in vivo temporal aspects of ACTH secretion: stimulatory actions of corticotrophin-releasing hormone and vasopressin in cattle. J. Vet. Med. A 54:7-14.

1. Pipet 50 µL of sample into labeled tube.
2. Add 450 µL of PBSG and vortex:
    a. Dissolve 8.17 g of NaCl, 0.856 g of NaH_2PO_4, 0.54 g of Na_2HPO_4 3.72 g of EDTA, and 0.1 g of thimerosal in 900 mL of double distilled water.
    b. Correct pH to 7.4.
    c. Add 1 g of gelatin to a 1 L bottle with the mixture from the previous step.
    d. Dissolve gelatin mixture on a hot plate with a magnetic stir at low to moderate heat for 2 h.
    e. PBSG is ready once cooled to room temperature.
    f. Store at 4° C.
3. Cover tubes and place in water bath at 70° C for 1 h.
4. Once samples are cooled to room temperature prepare the assay:
    a. Pipette 500 µL of standard and unknown into labeled tubes in triplicate.
    b. Add 100 µL of antibody to all tubes except those labeled T and N.
    c. Add 100 µL of trace to all tubes and vortex.

d. Incubate for 12 to 18 h at 4° C or incubate for 1 h at room temperature and then incubate at 4° C for 3 h.

5. Add 5 ml of Ecolume cocktail to mini-vials and label vials.
6. Continue procedure at 4° C.
7. Add 200 μL of mixed charcoal to all tubes except those labeled T.
    a. In a 200-mL beaker, mix 0.0625 g of Dextran Pharmacia T-70 with 0.525 g of Charcoal Norit SPXX and 100 mL of PBSG with the use of a magnetic stir bar.
    b. Store at 4° C for no more than 2 wk.
8. Shake racks of tubes and allow to set for 15 min.
9. Centrifuge for 10 min at 2282 x g.
10. Place tubes in cold room immediately after centrifuging or in ice.
11. Decant supernatant into mini-vials and count each vial for 1 min in beta counter.

APPENDIX B

SERUM ANTIBODY TITERS FOR *SALMONELLA* NEWPORT ELISA

References:

(Epitopix Biotechnology Company, Willmar, MN; Hermesch et al., 2008; Smith et al., 2014)

1. Coat 96-wells to a microtiter plate at a concentration of 250 ng per well, with Salmonella Newport–derived SRP antigen in carbonate-coating buffer (pH, 9.6).
2. Incubate plates at 4°C overnight (approximately 12 - 18 hr).
3. Empty plates and pat dry.
4. Block with 1% polyvinyl alcohol (PBS solution; 200 µL/well)
5. Cover plates and incubate at 37°C for 2 h.
6. Set two-fold dilutions (1:100 to 1:25,600) of serum samples in PBS solution.
7. Test these samples in duplicate.
8. At 37°C, cover plates and incubate for 1 h.
9. Wash plates with 0.05% PBS (Tween 20) solution 3 times.
10. Add 100 µL of horseradish-conjugated sheep anti-bovine IgG (diluted 1:1,600 in 1% sheep serum–Tween 20) to each well.
11. Cover plates and incubate at 37°C for 1 h.
12. Wash plates with Tween 20 solution 3 times.
13. Develop with 100 µL of 2,2′ azino-di-3-ethyl- benzthiazoline-6-sulfonate.
14. Measure optical absorbance with an ELISA reader at 405 to 490 nm.
15. Calculate sample-to-positive (S:P) ratios.

a. Calculate the average of the negative controls' optical density and subtract from all values as a reagent blank.
b. To factor out individual test variability, analyze the optical density (OD) reading and factor out a known positive and negative OD reading from the same test.
c. Average the sample duplicates and divide by the positive control average yielding the S:P titer.